

# ENTOMON

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## ENTOMON

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## Effect of temperature on the biotic potential of *Ceutorhynchus portulacae* (Coleoptera: Curculionidae) a potential natural enemy of *Portulaca oleraceae*

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**ABSTRACT:** *Ceutorhynchus portulacae* (Coleoptera: Curculionidae) was identified as a potential natural enemy of *Portulaca oleracea*, which is considered as a serious crop weed in many countries of the world. This present paper reports on the effect of constant temperature levels ranging from 10 to 45 °C on the survival and fecundity of *C. portulacae* adults. In respect of the biotic potential of the weevil, the temperature range of 25–30°C was found to be favourable. The implications of the findings in the biocontrol of the weed are discussed. © 2005 Association for Advancement of Entomology

**KEYWORDS:** *Portulaca oleraceae*, *Ceutorhynchus portulacae*, effect of temperature, biotic potential

### INTRODUCTION

A plant of South American origin, *Portulaca oleraceae* L. (Portulacaceae) commonly known as purslane weed, ranking as the world's ninth worst weed, is wide spread throughout the tropical, sub-tropical and temperate regions of the world (Holm *et al.*, 1977). In India, it is considered a serious weed in vegetables, vineyards, banana orchards, maize, cotton, groundnut, sorghum, sugarcane, sunflower and rice (Chadha *et al.*, 1995; Mandal, 1990). Based on detailed biological studies, the indigenous weevil *Ceutorhynchus portulacae* Marshall (Coleoptera: Curculionidae) was identified as a potential biocontrol agent that could be utilized for effective suppression of the weed (Ganga Visalakshy and Jayanth, 1997).

*P. oleraceae* grows under a wide range of climatic conditions in India, where the temperature varies from 0 °C in the cold season to as high as 49 °C during the hot season. Knowledge gained on the effect of temperature on survival and reproduction of the adults could assist in identifying the probable areas, where biological control of the purslane weed could be effective. Such information would be helpful to the workers

TABLE 1. Longevity and fecundity of *Ceutorhynchus portulacae* at different temperatures

| Temperature (°C) | Longevity                   | Fecundity<br>(No. of eggs/female) |
|------------------|-----------------------------|-----------------------------------|
| 10               | 32.25 (22–42) <sup>c</sup>  | –                                 |
| 15               | 37.25 (23–50) <sup>b</sup>  | –                                 |
| 20               | 53.65 (36–90) <sup>b</sup>  | 3                                 |
| 25               | 81.16 (81–117) <sup>a</sup> | 334.6 (208–438) <sup>a</sup>      |
| 30               | 66.50 (42–91) <sup>a</sup>  | 308.6 (206–387) <sup>a</sup>      |
| 35               | 18.76 (13–35) <sup>c</sup>  | 162.5 (142–235) <sup>b</sup>      |
| 40               | 9.84 (6–26) <sup>d</sup>    | 20.3 (18–24) <sup>c</sup>         |
| CD at 5%         | 19.91                       | 82.6                              |

Figures superscribed by the same letter are not significantly different.

in other countries of the world to explore the possibility of successful importation and release of *C. portulacae* for suppression of *P. oleraceae* in new habitats.

#### MATERIALS AND METHOD

Newly emerged adults of *C. portulacea* were released to oviposition jars (plastic jars of one litre capacity with an aerated lid) containing *P. oleraceae* bouquets. The bouquets were made from one-month-old *P. oleracea* plants grown in the glass house. Twigs of 10–15 cm length were cut, their bases were wrapped together with thick cotton and held by rubber band. The cotton swab was moistened as and when needed to prevent the exposed twigs from wilting. The bouquets exposed to the adults were replaced once in every two days. This process was repeated till the exposed adults died. Adults feed and lays egg on the leaves. The number of eggs laid, deeply embedded in the tissues were recorded with the help of a microscope.

The experiment was carried out at constant temperature levels of 10, 15, 20, 25, 30, 35, 40 and 45°C, 75–85% RH. Each treatment was replicated five times with three pairs of adults per replication.

#### RESULTS

Longevity studies indicated that temperature range of 25 and 30 °C to be optimum for the adults as significant survival was recorded at these temperatures followed by lower temperatures of 20 and 15 °C (Table 1). At 45 °C all the adults were found dead within 3–4 days. There was no variation in the longevity of females and males.

The temperature range of 25–40 °C was found to have no effect on the feeding and locomotion of the adults. However, at 10 °C and 15 °C most of the adults were inactive and did not feed. At 20 °C only a few adults were found active and their feeding marks could be observed. However, their activity was much reduced compared to the 25–30 °C range.

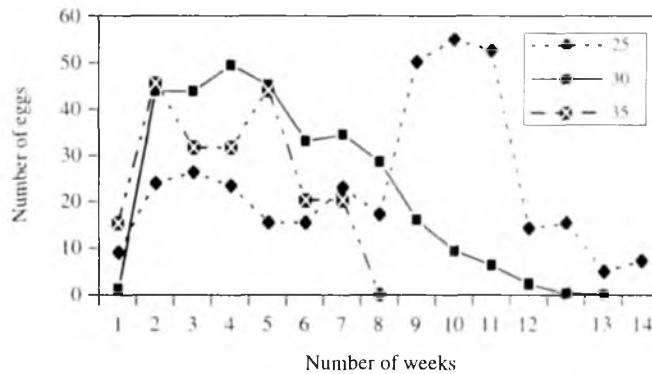


FIGURE 1. Age-specific fecundity of *C. portulacae* at different temperatures

No egg laying occurred at 10 °C and 15 °C. At 20 °C, only three eggs were laid, between the third and fourth week. At 25 °C, 335 eggs were laid (Table 1), with peak laying from eighth to tenth week (Fig. 1). Exposure of adults to temperature beyond 25 °C was found to reduce the egg laying capacity. An average of 273 eggs per female was obtained at 30 °C (Table 1) with peak egg period from third to seventh week. About 80% of the total eggs were laid by 7th week at 30 °C, while at 25 °C same percentage of eggs could be obtained only by 10th week (Fig. 1). At 35 °C, although high egg laying was recorded initially, due to early mortality, the average fecundity was only 162.45 eggs per female. The number of eggs per female further reduced to 20 at 40 °C. Females were found capable of laying eggs continuously with no post-oviposition period at all the temperatures studied the pre-oviposition period was 5–6 days at 25° and 30 °C and 2–3 days at 35 °C.

## DISCUSSION

The present study indicates that temperature influences the survival and normal activities of *C. portulacae* adults like feeding, locomotion and reproduction. Temperature levels of 25–30 °C were found to be optimum for the weevils. Under field conditions also, adults were found actively feeding and multiplying from May to November when the temperature was between 25 °C to 30 °C. The adults were found inactive under field conditions during the colder months of winter of December and January (14–22 °C) without egg laying and this observation is in conformity with the results of the present study. This indicates that in places which experience temperature below 20 °C the reproductive potential of *C. portulacae* will be reduced. Further, the long developmental period at 20 °C and below (Ganga Visalakshy, 2001) could result in delayed suppression of *P. oleraceae* by *C. portulacae*. Fall in temperature has been reported to cause a reduction in fecundity of *Zygogramma bicolorata*, *Cactoblastis cactorum* and *Agasicles hygrophilla*, potential biological control agents of *P. hystereophorus* and *Opuntia spp* and the Alligator weed *Alternanthera sp.*, respectively, thereby reducing

their effectiveness (Jayanth and Bali, 1993; Robertson and Hoffman, 1989; Stewart *et al.*, 1996). In places experiencing 40 °C and above the population build-up of *C. porulacae* may be inhibited.

Based on the present studies it is concluded that effective suppression of the weed by the weevil can be expected in regions experiencing atmospheric temperature within the range of 25–30 °C. This study also indicated the need for maintenance of the above temperature levels for successful mass rearing of the weevils.

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- Chadha, K. L., Leela, D. and Challa, P. (1995) *Weed management in Horticultural Crops and Plantation Crops*, Malhotra Publishing House: New Delhi, India.
- Ganga Visalakshy, P. N. and Jayanth, K. P. (1997) *Ceuthorhynchus portulacae* Marshall (Coleoptera: Curculionidae) a potential biological control agent of *Portulaca oleracea*. *Entomon* **22**: 150–151.
- Ganga Visalakshy, P. N. (2001) Bio-ecological studies on *Ceutorhynchus portulacae* and evaluation of its effectiveness in controlling the weed *Portulaca oleracea* Ph.D Thesis, Bangalore University, Bangalore.
- Holm, L. G., Plucknett, D. L., Pancho, J. V. and Herberger, J. P. (1977) The world's worst weeds. In: *Distribution and Biology*, Honolulu HI University Press: Hawaii, p609.
- Jayanth, K. P. and Bali, G. (1993) Temperature tolerance of *Zygogramma bicolorata* (Col: Chrysomelidae) introduced for biological control of *Parthenium hysterophorus* (Asteraceae) in India. *Journal of Entomological Research* **17**: 27–34.
- Robertson, H. G. and Hoffman, J. H. (1989) Mortality and life tables of *Cactoblastis cactorum* (Berg.) (Lepidoptera: Pyralidae) compared on two host plant species. *Bulletin of Entomological Research* **79**: 7–17.
- Mandal, R. C. (1990) *Weed, Weedicides and Weed Control—Principle and Practice*, Agro Botanicals Publishers.
- Stewart, C. A., Emerson, R. M. and Syrett, P. (1996) Temperature effects on the alligator weed flea beetle, *Agasicles hygrophilla*, imported for biological control in Newzealand. In: *Proceedings of 9th International Symposium on Biological Control of Weeds*, Stellenbosch, S. Africa, 19–26 January. Moram, H. C. and Hoffmann, J. A. (Eds).

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## Histo-biochemical studies on the male accessory sex glands of *Antheraea mylitta* (Drury) (Lepidoptera: Saturniidae) and the effects of juvenile hormone and ecdysone

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**ABSTRACT:** In *Antheraea mylitta* a pair of long, coiled, tubular male accessory sex glands (MASG) are extended into the abdominal cavity and open into the duplex ejaculatory ducts. The wall of the MASG is composed of a layer of columnar epithelial cells and the lumen is filled with secretory material in the newly emerged male moth. The histochemical studies revealed the presence of abundant quantity of DNA in the nuclei, RNA in the nuclei and cytoplasm suggesting active secretory activity soon after emergence of the adult male. The histochemical tests suggest that the secretory material accumulated in the cells and lumen of the MASG is a mixture of protein, carbohydrate and lipid. The biochemical analysis showed that the secretory material of MASG is composed of a large quantity of protein and a little amount of carbohydrate and lipid. The maximum level of DNA, RNA, protein, carbohydrate and lipid concentration in MASG was noticed in the newly emerged male moth which was reduced gradually in the old moths suggesting gradual reduction in the secretory activity of the MASG. The SDS-PAGE separated in-all 22 protein bands ranging from 19.17 to 205.000 in molecular weight. Topical application of JH- III on the newly emerged male moths caused significant increase while that of  $\beta$ -ecdysone showed reduction in the total protein concentration of MASG.

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**KEYWORDS:** *Antheraea mylitta*, juvenile hormone, ecdysone, male accessory sex glands

### INTRODUCTION

Development, structure and functions of the male accessory sex glands (MASG) in the insects have been reviewed (Leopold, 1976; Chen, 1984; Happ, 1984, 1992; Gillott, 1995). Their secretion is used for the formation of the spermatophore and in various reproductive functions such as a source of seminal fluid, sperm activation, sperm

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capacitation, sperm nourishment and stimulation of oviposition (Gillott, 1988, 1995). The chemical nature of MASG secretion in insects has been analyzed as the mixture of proteins, mucopolysaccharides and lipids (Chen, 1984; Gillott, 1988). Gillott and Venkatesh (1985) and Sridevi (1987) have reported the protein pattern of the MASG secretion. The present investigation deals with structure, analysis of chemical nature and the effect of JH and ecdysone on MASG in the tasar silkworm, *Antheraea mylitta*.

## MATERIAL AND METHODS

The cocoons were brought from Tasar Research Training Centre, Dawadipar, Bhandara (India) and kept in the laboratory till the emergence of adults (Jolly *et al.*, 1979). The adult males were separated and used for the present study.

### Histology

The MASG were dissected in saline from the newly emerged adults and fixed in aqueous Bouin's fluid for 18–24 h. Paraffin sections were cut at 4  $\mu\text{m}$  and stained with Heidenhain's iron haematoxylin-eosin.

### Histochemical methods

The glands were fixed in Carnoy's fixative or 10% formalin for 6–8 h, dehydrated in ethanol, cleared in xylene and embedded in paraffin wax (60–62 °C). Four  $\mu\text{m}$  thick sections were treated with Feulgen reaction for DNA, Brachet's Toluidine blue test for RNA, Mazia *et al.*, mercury bromophenol blue (Hg-BPB) reaction for protein and Hotchkiss's periodic acid Schiff's reagent (PAS) techniques for carbohydrates. For lipid, Chieffelle and Putt's Sudan black-B (SBB) technique was applied on the 10% calcium formol fixed frozen sections (Pearse, 1968).

### Biochemical estimation

The MASG were removed from 0- (newly emerged), 1 and 2-day-old adults. The MASG were separated, tracheae and fat body were removed and homogenized at 0 °C for 5 min in different volumes of ice-cold distilled water, Ringer's solution and 0.25 M sucrose solution separately. For lipid, the MASG were homogenized in 20 ml of chloroform, methanol (2:1 v/v) containing 0.01% butylated hydroxy-toluene (BHT) as an antioxidant and centrifuged at 3000 rpm for 15 min. Total concentration of DNA and RNA was estimated by Burton's Diphenylamine (Searcy and MacInnis, 1970a) and Dische-Orcinol (Searcy and MacInnis, 1970b) methods respectively. The procedure of Lowry *et al.* (1951) was followed for the estimation of total protein. Total lipid was estimated using the method of Frings and Dunn (Tietz, 1966). The method of Dubois *et al.*, was used for the estimation of total carbohydrates (Neufeld and Ginsburg, 1966).

### Electrophoresis

The 1 mm 3% stacking gel (pH 6.8) was followed by a 10 cm 10% separating gel (pH 8.8) with 1% SDS. MASG from 0, 1 and 2-day-old adult male moths were dissected out and cut into pieces, homogenized and centrifuged as mentioned above and the supernatant was used as the sample. 50  $\mu$ l of clear supernatant was mixed with 50  $\mu$ l of treatment buffer (Tris-2.5 ml, pH 6.8, SDS-4 ml, Glycerol-2 ml, 2-Mercaptoethanol-1 ml, distilled water-0.5 ml and a pinch of Bromophenol blue). The samples were heated for 5 minutes in a water bath. The mixture was cooled and its 25  $\mu$ l, 30  $\mu$ l, 35  $\mu$ l quantity was separately applied onto the top of the gel. Standard wide range molecular weight marker protein was also run together. The gel was stained with Coomassie brilliant blue for 2 h and destaining was done with a mixture of methanol-acetic acid-distilled water until the bands on the gel became clear. The molecular weight of the protein bands with regard to the marker proteins was estimated with the help of the Densitometer (Pendram, 2002).

### Application of JH III and $\beta$ -ecdysone

The 200  $\mu$ g JH III and 20  $\mu$ g  $\beta$ -ecdysone (Sigma, U. S. A.) dissolved separately in 20  $\mu$ l of cold acetone were applied topically on the dorsal surface of the newly-emerged adults, with the help of Hamilton's CR-700 constant syringe. Equal number of adults of another group were treated with cold acetone only to serve as control. After the intervals of 24 and 48 h, the MASG were dissected gently from 6–10 experimental and equal number of control insects, homogenized separately and supernatant was used for estimation of total protein concentration of MASG.

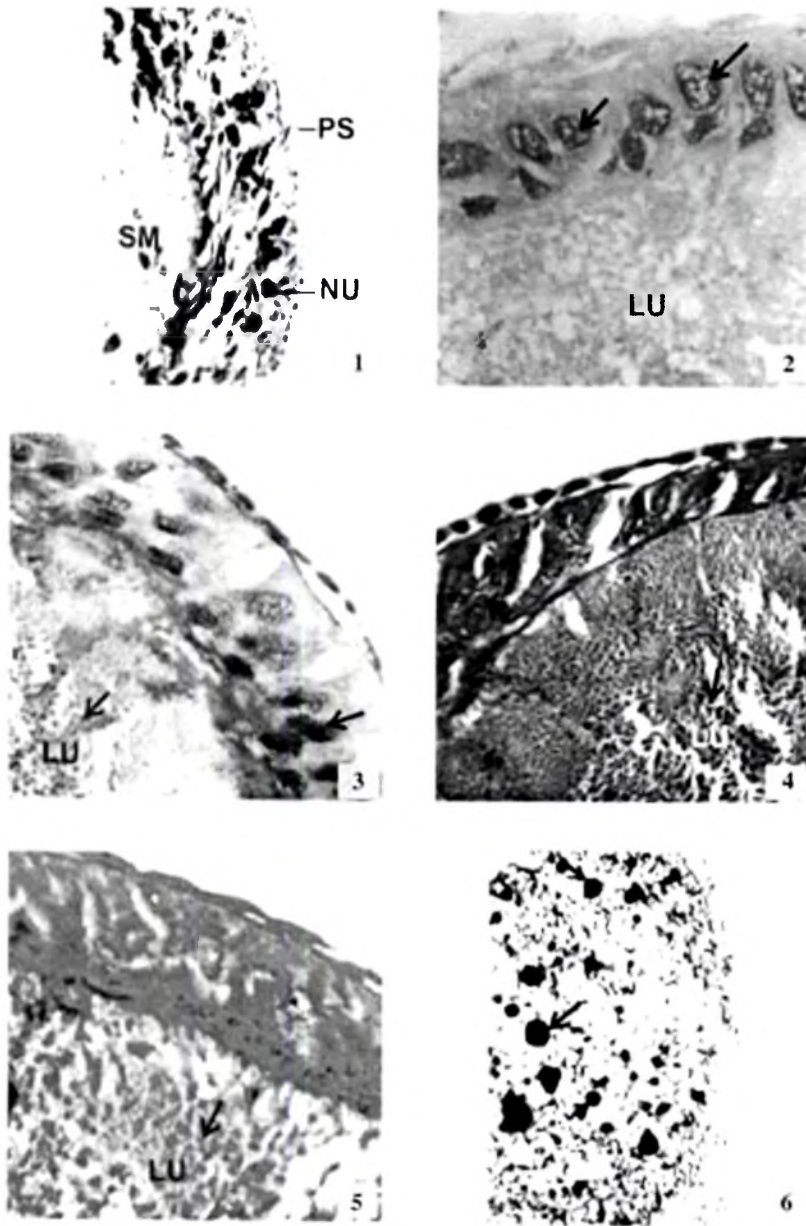
## RESULTS

### Histology

The MASG are paired,  $21.4 \pm 0.8$  mm long, coiled and tubular structures. They open into the ejaculatory duplex ducts. The wall of the MASG is composed of an outer peritoneal sheath and an inner layer of epithelium. The MASG wall is devoid of muscle layer. The epithelium consists of the tall columnar cells with prominent nuclei and dense cytoplasm. The nuclei are elongated and measure about 12  $\mu$ m in diameter. The secretion of the epithelial cells is discharged into the lumen in the form of fine secretory droplets. Internal surface of the epithelium is devoid of cuticular intima suggesting the MASG as the mesadenia (Fig. 1).

### Histochemistry

Intense reaction in the nuclei of epithelial cells was observed after the Feulgen and Toluidine blue tests suggesting presence of abundant quantity of DNA and RNA (Figs. 2 and 3). The cytoplasmic inclusion in the epithelial cells as well as secretory material in the lumen was reacted intensely with the Hg-BPB, PAS and SBB tests suggesting protein, carbohydrates and lipids in the secretory material of MASG (Figs. 4–6).



FIGURES 1–6: Histological and histochemical staining of the transverse section passing through accessory gland showing: Fig. 1. Histologicla structure: PS–outer peritoneal sheath, NU–nucleus, LU–lumen, SM–secretory material, X240 FeH-E; Fig. 2. Feulgen reaction showing DNA (arrow) X240; Fig. 3. Toluidine blue showing RNA (arrow) X240; Fig. 4. Mercury bromophenol blue showing protein (arrow) X240; Fig. 5. Periodic acid Schiff's showing carbhydrate (arrow) X240; Fig. 6. Sudan-black B showing lipids (arrow) X128.

TABLE 1. Biochemical analysis of MASG

| Substance                                 | Age of the adult male (days) |                  |                  |
|---|------------------------------|------------------|------------------|
|   | 0                            | 1                | 2                |
| DNA ( $\mu\text{g}/\text{mg}$ )           | $11.28 \pm 0.40$             | $8.34 \pm 0.34$  | $5.92 \pm 0.40$  |
| RNA ( $\mu\text{g}/\text{mg}$ )           | $9.85 \pm 0.07$              | $6.86 \pm 0.05$  | $3.5 \pm 0.07$   |
| Protein ( $\mu\text{g}/\text{mg}$ )       | $305.3 \pm 2.15$             | $300.8 \pm 1.54$ | $288.8 \pm 7.68$ |
| Carbohydrate. ( $\mu\text{g}/\text{mg}$ ) | $0.43 \pm 0.01$              | $0.25 \pm 0.01$  | $0.23 \pm 0.01$  |
| Lipids ( $\text{mg}/100\text{ml}$ )       | $560 \pm 0.07$               | $519 \pm 0.06$   | $440 \pm 0.06$   |

$\pm$ —SE of mean value.

TABLE 2. Protein concentration ( $\mu\text{g}/\text{mg}$ )

| Treatment days | JH III              |                 | Ecdysone          |                 |
|----------------|---------------------|-----------------|-------------------|-----------------|
|                | Experimental        | Control         | Experimental      | Control         |
| 1              | $*577.6 \pm 15.36$  | $300.8 \pm 1.5$ | $**222 \pm 1.8$   | $300.8 \pm 1.5$ |
| 2              | $**577.6 \pm 15.36$ | $288.8 \pm 7.6$ | $**207.1 \pm 1.4$ | $288.8 \pm 7.6$ |

\* $P = 0.05$ , \*\* $P = 0.01$ ,  $\pm$ —SE of mean value

### Biochemical observations

In the 0 (newly emerged), 1 and 2 day old adults the DNA, RNA, protein, carbohydrate and lipid showed reduction in their quantity gradually (Table 1).

### Electrophoretic separation of proteins

SDS-PAGE analysis of the MASG secretions showed separation of 22 protein bands ranging from 19.1 to 205.000 in molecular weight (Fig. 7).

### Application of JH-III and $\beta$ -ecdysone on protein concentration

The topical application of JH-III caused significant rise in the concentration of protein in MASG than that in the control insects after a period of 1 and 2 days (Table 2). Although a decrease in total protein concentration was observed in 1 and 2-day old control insects, the elevated concentration of total proteins in the JH III treated insects after 1 and 2 days interval remained constant. On the other hand, treatment of  $\beta$ -ecdysone resulted significant reduction in protein concentration after 1 and 2 days from that in the control insects.

## DISCUSSION

In the newly emerged *Antheraea mylitta* the MASG are evident as a pair of long coiled tubular structures composed of columnar epithelium covered externally with peritoneal sheath and enclosing internally a large lumen but without internal cuticular intima suggesting their mesodermal origin. The male accessory sex glands of the mesodermal origin are also observed in many insects (Chen, 1984). The chemical

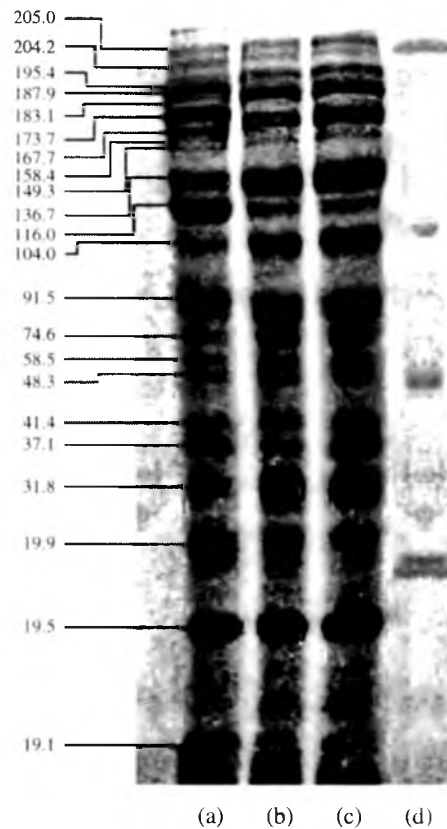


FIGURE 7. SDS-PAGE analysis of various protein bands of the male accessory sex gland: (MASG) (a) newly emerged adult, (b) one day old adult, (c) two day old adult and (d) standard molecular weight marker proteins (205-6.500 kD).

composition of secretory material of MASG in *Antheraea mylitta* is a mixture of protein, carbohydrate and lipid similar to that in other insects (De Loof and Lagasse, 1972; Vijayalekshmi and Adiyodi, 1973; Ranganathan *et al.*, 1984). The MASG showed maximum concentration of total DNA, RNA, protein, carbohydrate and lipid in the newly emerged males which was reduced gradually with the advancement of age suggesting initiation of vigorous secretory activity soon after emergence of an adult male from a cocoon and its gradual reduction in the older insects (Kaulenas, 1992). The MASG secretion containing large number of proteins has been reported in Lepidoptera i.e., 49 to 50 in *Spodoptera litura* (Sridevi, 1987) and 32 in *Opisina arenosella* (Santhosh Babu, 1995). Electrophoretic separation of MASG in *Antheraea mylitta* showed in-all 22 distinct protein bands varying from each other in their molecular weight. The MASG secreting a large number of proteins and performing various functions has been investigated by several workers (Chen, 1984, 1996; Gillott, 1988, 1995; Happ, 1992).

Topical application of juvenile hormone caused significant rise in protein in MASG of *Antheraea mylitta* supporting the earlier workers suggesting stimulatory effect of JH III on protein synthesis (Odhiambo, 1966; De Loof and Lagasse, 1972; Gillott and Friedel, 1976; Koeppe *et al.*, 1985; Couche and Gillott, 1987; Cheesman and Gillott, 1989; Mane and Subrahmanyam, 2000). In *Oryctes rhinoceros*, *in vitro* development and secretory activity of male accessory gland occurred after the corpus allatum added to a culture (Jacob, 1993).

In *Antheraea mylitta*,  $\beta$ -ecdysone exerted an inhibitory action on MASG. In *Tenebrio molitor* and *Bombyx mori*, ecdysteroids were found stimulating the development of MASG at the pupal stage but acting adversely during the adult stage (Shinbo and Happ, 1989; Yaginuma and Happ, 1989). Male accessory gland rudiment in *Oryctes rhinoceros* showed only proliferation of cells *in vitro* in presence of 20-hydroxyecdysone but no tubular development of the glands and thus indicating an inhibitory action (Jacob, 2001).

The present observations, therefore, strongly suggest that the JH is stimulating while the  $\beta$ -ecdysone is inhibiting the secretory activity of MASG in the tasar silkworm, *Antheraea mylitta*.

#### REFERENCES

- Chen, P. S. (1984) The functional morphology and biochemistry of insect male accessory glands and their secretions. *Annual Review of Entomology* **29**: 233–255.
- Chen, P. S. (1996) The accessory gland proteins in *Drosophila*: structural, reproduce and evolutionary aspects. *Experientia* **52**: 503–10.
- Cheesman, M. T. and Gillott, C. (1989) Long hyaline gland discharge and multiply spermatophore formation by the male grasshopper, *Melanoplus sanguinipes*. *Physiological Entomology* **14**: 257–264.
- Couche, G. A. and Gillott, C. (1987) Development of secretory activity in the long hyaline gland of the male migratory grasshopper, *Melanoplus sanguinipes* (Faber) (Orthoptera: Acrididae). *International Journal of Insect Morphology and Embryology* **16**: 355.
- De Loof, A. and Lagasse, A. (1972) The ultrastructure of the male accessory reproductive glands of the Colorado beetle. *Z. Zellforsch.* **130**: 545–552.
- Gillott, C. (1988) Arthropoda-Insecta. In: *Reproductive Biology of Invertebrates Vol 3*, (Accessory Sex Glands). Adiyodi, K. G. and Adiyodi, R. G. (Eds). Oxford and IBH: New Delhi, pp. 319–471.
- Gillott, C. (1995) Insect male mating systems. In: *Insect Reproduction*, Leather, S. R. and Hardie, J. (Eds). CRC Press: New York, 33–55.
- Gillott, C. and Friedel, T. (1976) Development of accessory reproductive glands and its control by the corpus allatum in adult *Melanoplus sanguinipes*. *Journal of Insect Physiology* **22**: 365–372.
- Gillott, C. and Venkatesh, K. (1985) Accessory reproductive glands of the male migratory grasshopper. Accumulation of secretory proteins in the *Melanoplus sanguinipes*: A development study. *Journal of Insect Physiology* **31**: 195–204.
- Happ, G. M. (1984) Sturcture and development of male accessory glands in insect. In: *Insect Ultrastructure*, King, R. C. and Akai, H. (Eds). Plenum Press: New York, 365, vol 2.
- Happ, G. M. (1992) Maturation of the male reproductive system and its endocrine regulation. *Annual Review of Entomology* **37**: 303.
- Jacob, M. (1993) Endocrines in *in vitro* development of male accessory sex glands of *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). *Indian Journal of Comparative Animal Physiology* **11**: 50–53.

- Jacob, M. (2001) Organ culture: *in vitro* development of male accessory sex glands of *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae). *Entomon* 26 (Spl. Issue): 222–225.
- Jolly, M. S., Sen, S. K., Sonwalkar, T. N. and Prasad, G. K. (1979) *Non-Mulberry silk*. *FAO. Agriculture Service Bulletin* 29: 1–178.
- Kaulenas, M. S. (1992) Insect accessory reproductive structures: Function Structure and Development. *Zoophysiology* 31: 224.
- Koepe, J. K., Fuchs, M. S., Chen, T. T., Hunt, L. M., Kovalick, G. E. and Briers, T. (1985) The role of juvenile hormone in reproduction. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology Vol 8*, Kerkut, G. A. and Gilbert, L. I. (Eds). Pergamon Press: Oxford, UK.
- Leopold, R. A. (1976) The role of male accessory glands in insect reproduction. *Annual Review of Entomology* 21: 199–222.
- Lowry, L. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 219: 65–275.
- Mane, A. and Subrahmanyam, B. (2000) Regulation of protein and RNA synthesis in male accessory glands of *Spodoptera litura*. (Faber.) (Lepidoptera: Noctuidae) by juvenile hormone and jenvnoids. *Entomon* 25: 1–13.
- Neufeld, E. F. and Ginsburg, V. (1966) *Methods in Enzymology*, Academic Press: New York and London, p711.
- Odhiambo, T. R. (1966) Site of action of the corpus allatum hormone at the cellular level in *Schistocerca gregaria*. *Acta Trop.* 23: 264.
- Pearse, A. G. E. (1968) *Histochemistry, Theoretical and Applied*, 3rd edn. 1. Churchill-Livingston: London.
- Pendam, V. R. (2002) Studies on the male reproductive system in the tropical tasar silk-worm, *Antheraea mylitta* (D) (Lepidoptera: Saturniidae) *Ph.D. Thesis*, Nagpur University, Nagpur, India.
- Ranganathan, L. S., Sriramulu, V., Balasundaram, D. and Sridharan, G. (1984) Role of glucose and glycogen in the accessory reproductive gland and sperm transfer in *Aspongopus janus*. *Current Science* 53: 713–714.
- Santhosh Babu, P. B. (1995) Histology and secretory activity of accessory reproductive organs in male *Opisina arenosella* Walker (Lepidoptera: Xyloryctinae). *Entomon* 20: 209–213.
- Searcy, D. G. and MacInnis, A. J. (1970a) Biochemical estimation of DNA by Burton's Diphenylamine method. *Journal of Biochemistry* 62: 315–323.
- Searcy, D. G. and MacInnis, A. J. (1970b) Biochemical estimation of RNA by Dische-Orcinal method. *Journal of Biochemistry* 62: 15–323.
- Shinbo, H. and Happ, G. M. (1989) Effect of ecdysteroids on the growth of the post-testicular reproductive organs in the silkworm, *Bombyx mori*. *Journal of Insect Physiology* 35: 855.
- Sridevi, R. N. (1987) Studies on the nucleic acids, proteins and changes in some enzymatic activities in the testis, fat body and accessory glands and their hormonal regulation during development in the male of *Spodoptera litura* (Lepidoptera: Noctuidae) *Ph.D. Thesis*, University of Hyderabad, India, (submitted).
- Tietz, N.W. (1966) *Fundamental of Clinical Chemistry*, Press of W. B. Saunders Co: p1176.
- Vijayalekshmi, V. R. and Adiyodi, K. G. (1973) Accessory sex glands of male *Periplaneta americana* L. Part. III. Histochemistry of the mushroom-shaped and conglobate glands. *Indian Journal of Experimental Biology* 11: 521–524.
- Yaginuma, H. and Happ, G. M. (1989) 20-Hydroxy ecdysone acts in the male pupa to commit accessory glands toward trehalase production in the adult mealworm beetle (*Tenebrio molitor*). *General and Comparative Endocrinology* 73: 173.

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## Differential susceptibility of silkworm, *Bombyx mori* L. races to white muscardine disease caused by *Beauveria bassiana* (Bals.) Vuill

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**ABSTRACT:** The susceptibility of various silkworm races viz., multivoltine Pure Mysore, APM<sub>1</sub> and APS<sub>8</sub> were screened for resistance to entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. Freshly moulted fifth instar larvae of all races were inoculated with various aliquots of the fungal spore suspension (10<sup>1</sup> to 10<sup>9</sup> conc. of spore/ml). The LC<sub>50</sub> values and other parameters showed that bivoltine race, APS<sub>8</sub> was most susceptible to *Beauveria bassiana* compared to the other races.

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**KEYWORDS:** *Beauveria bassiana*, *Bombyx mori*, lethal concentration, mortality, Pure Mysore (PM), APM<sub>1</sub>, APS<sub>8</sub>

### INTRODUCTION

The white muscardine disease caused by an entomopathogenic fungus, *Beauveria bassiana* is devastating the sericulture industry. Even though the loss due to muscardine infection is generally very low to the extent of about 90% but it may be observed as high as 70% or more which may cause total failure of the crop in several cases. The loss incurred due to white muscardine disease ranges from 5 to 50% in different countries. (Jhansi Lakshmi, 2003). Wide host range, faster rate of spread of pathogen, improper disinfection, poor rearing management and poor hygienic conditions are the main reasons attributed for the outbreak of white muscardine disease (Mallikarjuna, 1998). In spite of modern rearing techniques, the farmers find it difficult to produce

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desirable quantities of high quality cocoons, if white muscardine disease attacks the silkworms.

The visual symptoms of white muscardine affected silkworms appear mostly at the later stages of the larval period. The disease becomes manifest only after it is firmly established in the body of the worm and gives no chance for eliminating the disease by killing the pathogen in the short period available before the worm reaches the terminal spinning phase. Owing to white muscardine disease, the growth and economic traits of silkworm larvae have significantly effected, moreover adverse effects and severe damage occurred to the internal tissues and organs. Several workers indicated the differential sensitivity of some silkworm races to the pathogen, *Beauveria bassiana* (Reddy, 1978; Jayaramaiah, 1981; Chinnaswami, 1983; Raghavaiah and Jayaramaiah, 1989). However, there are limited reports available on the lethal concentration of the fungus to different races of fifth instar silkworm larvae at different time intervals. Hence, a study was systematically designed to evaluate the fungal spore concentration versus susceptibility of various races of *Bombyx mori* larvae.

#### MATERIALS AND METHODS

The popular multivoltine race, Pure Mysore (PM), and newly evolved multivoltine APM<sub>1</sub> and bivoltine APS<sub>8</sub> races were selected for the present study. The larvae were reared following the methods of Krishnaswamy (1978, 1979). *Beauveria bassiana* was isolated from diseased worms and pure cultures were maintained as per the procedures of Govindan *et al.* (1998). Nine spore concentrations of the fungus *viz.*, 10<sup>1</sup> to 10<sup>9</sup> spore/ml suspension with four replications each were used for infecting the larvae. In each replication, 50 larvae were taken in a tray and were sprayed with the respective spore suspensions. The contaminated fifth instar larvae were reared under slightly high humid conditions (25 ± 1 °C Temperature & 70–80% RH) for effective growth of the fungus and all the worms were reared as per the standard procedures (Krishnaswamy, 1978). Mortality data were subjected to probit analysis and regression analysis (Finney, 1971; Suryanarayana Murty *et al.*, 2002).

#### RESULTS AND DISCUSSION

It was found that the susceptibility of the three races of *Bombyx mori* to the fungal attack was different for each race. It was noticed that Pure Mysore race was more resistant compared to the other two races. When the larvae of all the races *viz.*, Pure Mysore, APM<sub>1</sub>, and APS<sub>8</sub> were treated with 1 × 10<sup>1</sup> spores per ml of *Beauveria bassiana*, no mortality occurred (Table 1). The LC<sub>50</sub> for Pure Mysore was with higher inoculum *i.e.*, when treated with 10<sup>7</sup> spore conc./ml (Table 2). The multiple correlation coefficient (*R*<sup>2</sup>) value of the 4th, 5th and 6th days of treatment of fifth instar Pure Mysore larvae were 0.936, 0.930 and 0.928 respectively (Table 3).

When the APM<sub>1</sub> larvae treated with 1 × 10<sup>3</sup> spores per ml, mortality rates of 6%, 10% and 24% were noticed, and highest mortality rates of 66%, 86% and 100% were found in treatment with 1 × 10<sup>9</sup> spore concentration on the fourth, fifth and sixth days.

TABLE 1. Per cent mortality of silkworm larvae versus spore concentration (spores/ml) of *Beauveria bassiana*

| Breed            | Days after treatment | No. of larvae used | % mortality versus concentration of spores |                 |                 |                 |                 |                 |                 |                 |                 |
|------------------|----------------------|--------------------|--|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  |                      |                    | $1 \times 10^1$                            | $1 \times 10^2$ | $1 \times 10^3$ | $1 \times 10^4$ | $1 \times 10^5$ | $1 \times 10^6$ | $1 \times 10^7$ | $1 \times 10^8$ | $1 \times 10^9$ |
| Pure Mysore      | 4                    | 50                 | 0  | 0               | 0               | 10              | 22              | 34              | 46              | 56              | 62              |
|                  | 5                    | 50                 | 0  | 0               | 6               | 22              | 38              | 54              | 62              | 72              | 82              |
|                  | 6                    | 50                 | 0  | 2               | 12              | 40              | 62              | 94              | 100             | 100             | 100             |
| APM <sub>1</sub> | 4                    | 50                 | 0  | 0               | 6               | 14              | 28              | 38              | 52              | 62              | 66              |
|                  | 5                    | 50                 | 0  | 0               | 10              | 24              | 42              | 62              | 66              | 74              | 86              |
|                  | 6                    | 50                 | 0  | 8               | 24              | 44              | 68              | 100             | 100             | 100             | 100             |
| APS <sub>8</sub> | 4                    | 50                 | 0  | 0               | 10              | 22              | 44              | 58              | 66              | 74              | 78              |
|                  | 5                    | 50                 | 0  | 6               | 18              | 40              | 62              | 68              | 82              | 94              | 100             |
|                  | 6                    | 50                 | 0  | 12              | 36              | 66              | 80              | 100             | 100             | 100             | 100             |

Pure Mysore = Multivoltine; APM<sub>1</sub> = Multivoltine; APS<sub>8</sub> = BivoltineTABLE 2. Lethal concentration (LC<sub>50</sub>) of *Beauveria bassiana* to different races of silkworm, *Bombyx mori* larvae

| Races            | Day | LC <sub>50</sub>               | Lower Fiducial Level          | Upper Fiducial Level          | Regression Coefficient | Intercept             | t-value                 |
|------------------|-----|--------------------------------|-------------------------------|-------------------------------|------------------------|-----------------------|-------------------------|
| Pure Mysore      | 4   | $3.68 \times 10^7$<br>(7.565)* | $1.42 \times 10^7$<br>(7.151) | $1.18 \times 10^8$<br>(8.071) | 0.3809<br>$\pm 0.038$  | -2.882<br>$\pm 0.255$ | 9.986<br>(Significant)  |
|                  | 5   | $1.86 \times 10^6$<br>(6.268)  | $8.41 \times 10^5$<br>(5.925) | $4.32 \times 10^6$<br>(6.635) | 0.4123<br>$\pm 0.034$  | -2.584<br>$\pm 0.216$ | 11.817<br>(Significant) |
|                  | 6   | $2.44 \times 10^4$<br>(4.388)  | $1.47 \times 10^4$<br>(4.166) | $4.10 \times 10^4$<br>(4.610) | 0.884<br>$\pm 0.080$   | -3.882<br>$\pm 0.365$ | 10.997<br>(Significant) |
| APM <sub>1</sub> | 4   | $1.45 \times 10^7$<br>(7.161)  | $5.63 \times 10^6$<br>(6.751) | $4.44 \times 10^7$<br>(7.647) | 0.360<br>$\pm 0.034$   | -2.575<br>$\pm 0.223$ | 10.447<br>(Significant) |
|                  | 5   | $9.16 \times 10^5$<br>(5.962)  | $4.22 \times 10^5$<br>(5.625) | $2.06 \times 10^6$<br>(6.314) | 0.419<br>$\pm 0.035$   | -2.496<br>$\pm 0.209$ | 12.152<br>(Significant) |
|                  | 6   | $1.08 \times 10^4$<br>(4.033)  | $6.34 \times 10^3$<br>(3.802) | $1.85 \times 10^4$<br>(4.267) | 0.809<br>$\pm 0.072$   | -3.263<br>$\pm 0.302$ | 11.303<br>(Significant) |
| APS <sub>8</sub> | 4   | $1.32 \times 10^6$<br>(6.122)  | $3.49 \times 10^5$<br>(5.543) | $5.78 \times 10^6$<br>(6.762) | 0.391<br>$\pm 0.033$   | -2.394<br>$\pm 0.203$ | 11.808<br>(Significant) |
|                  | 5   | $6.55 \times 10^4$<br>(4.816)  | $3.31 \times 10^4$<br>(4.520) | $1.29 \times 10^5$<br>(5.109) | 0.517<br>$\pm 0.040$   | -2.491<br>$\pm 0.210$ | 12.917<br>(Significant) |
|                  | 6   | $3.66 \times 10^3$<br>(3.563)  | $2.14 \times 10^3$<br>(3.330) | $6.22 \times 10^3$<br>(3.794) | 0.814<br>$\pm 0.074$   | -2.898<br>$\pm 0.282$ | 11.048<br>(Significant) |

\*Figures in parantheses indicate the log-transformed value.

TABLE 3. Mortality of different races of silkworm, *Bombyx mori* larvae in different days

| Races            | Day | $R^2$ | SE     | F      | df  | P-value<br>( $P < 0.01$ ) |
|------------------|-----|-------|--------|--------|-----|---------------------------|
| Pure Mysore      | 4   | 0.936 | 0.571  | 102.46 | 1.7 | Significant               |
|                  | 5   | 0.930 | 0.537  | 93.15  | 1.7 | Significant               |
|                  | 6   | 0.928 | 0.550  | 90.17  | 1.7 | Significant               |
| APM <sub>1</sub> | 4   | 0.926 | 0.5171 | 87.72  | 1.7 | Significant               |
|                  | 5   | 0.922 | 0.5340 | 82.98  | 1.7 | Significant               |
|                  | 6   | 0.907 | 0.4964 | 68.32  | 1.7 | Significant               |
| APS <sub>8</sub> | 4   | 0.923 | 0.5301 | 84.89  | 1.7 | Significant               |
|                  | 5   | 0.903 | 0.5008 | 65.88  | 1.7 | Significant               |
|                  | 6   | 0.900 | 0.5469 | 54.32  | 1.7 | Significant               |

$R^2$  = Multiple correlation coefficient; SE = Standard Error; F = F-test value; df = degrees of freedom; P = significance levels.

(Table 1). In case of multivoltine, APM<sub>1</sub> the LC<sub>50</sub> was 10<sup>6</sup> spore conc./ml (Table 2). The multiple correlation coefficient ( $R^2$ ) values for the APM<sub>1</sub> race on 4th, 5th and 6th days of treatment were 0.926, 0.922 and 0.907 respectively (Table 3).

In the case of APS<sub>8</sub>, the mortality rates of 10%, 18% and 36% were found when treated with  $1 \times 10^3$  spores per ml on fourth, fifth and sixth days and highest mortality of 100% was obtained for  $1 \times 10^9$  concentration on the fifth and sixth days (Table 1). While the LC<sub>50</sub> for bivoltine, APS<sub>8</sub> was with a conc. of 10<sup>5</sup> spore/ml (Table 2). The  $R^2$  values for this race obtained were 0.923, 0.903 and 0.900 on the 4th, 5th and 6th days of fungal treatment (Table 3).

Highest larval mortality with lowest LC<sub>50</sub> values was found on the sixth day while the lowest mortality with highest LC<sub>50</sub> values on the fourth day of inoculation was noticed in all the silkworm races (Pure Mysore, APM<sub>1</sub>, & APS<sub>8</sub>). The effective time for complete larval mortality (100%) was found on the sixth day of infestation in case of Pure Mysore race where as it was fifth day in case of APM<sub>1</sub> and APS<sub>8</sub> races.

The above observations revealed that Pure Mysore race was comparatively less susceptible to *Beauveria bassiana* than APM<sub>1</sub> and APS<sub>8</sub> races. It was also statistically proved that the multiple correlation coefficient ( $R^2$ ) values for different days of treatment for three silkworm races were more than 0.90 which indicates the applicability of probit and regression analysis (Finney, 1971; SPSS version 5.0 and 10.0) in determining LC<sub>50</sub> values for fungus toxicological studies. The  $R^2$  values were significant and they were gradually decreased in the experiment when the values for Pure Mysore race were compared to the values of APM<sub>1</sub> and APS<sub>8</sub> ( $P < 0.01$ ; Table 3).

According to the Werren (1997), symbiotic associations between microorganisms and higher eucaryotes are extremely common, and they range from mutualistic (beneficial) to commensal (neutral) and parasitic (harmful). The silkworm, *Bombyx mori* is prone to various pests and diseases. Several predisposing factors play

an important role in causing disease epizootics. The occurrence and spread of silkworm diseases are closely related to the constitution of the worms, pathogen and environmental conditions. Early instar silkworms are more susceptible to infectious diseases than late instars. However, white muscardine is serious in later stages. According to Govindan and Devaiah (1995), the infection process is determined by the balance between the devices of aggression of the pathogen and its virulence. It also depends upon the mechanism of defense, the genetic resistance, susceptibility and immunity of the host insect and the stress exerted by the environment or various other agents.

The present study revealed that the larval mortality decreased with increase in dilution of the spore suspension. The extent of LC<sub>50</sub> values of *Beauveria bassiana* for different silkworm races was found to vary due to the susceptibility of the host, virulence of the specific pathogen, biotic and abiotic factors and immunity of the host and host-pathogen relationship. However, the susceptibility might have direct relationship to the body weight as well as larval duration, that is, heavier races with lesser larval duration (APM<sub>1</sub> & APS<sub>8</sub>) are more susceptible to the fungus attack as against the light race with longer larval duration (Pure Mysore). Similar observations were earlier noticed by Paillot (1930), Steinhaus (1949) and Govindan *et al.* (1998) and they reported that the silkworm races exhibited acquired immunity to the white muscardine disease.

Earlier reports indicated that multivoltine races have shown less susceptibility to white muscardine disease than the bivoltine races and their hybrids at a spore concentration of 10<sup>2</sup> spores/ml. (Anonymous, 1973; Raghavaiah and Jayaramaiah, 1989, 1990). Whereas, in the present investigation, it was found that the bivoltine race i.e., APS<sub>8</sub> exhibited marked susceptibility compared to multivoltine races viz., Pure Mysore and APM<sub>1</sub> using a range of aliquots of spore concentration (10<sup>1</sup> to 10<sup>9</sup>). This investigation has also projected the statistical analysis of variance and probit analysis, besides determining the LC<sub>50</sub> values.

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#### REFERENCES

- Anonymous, (1973) *Silkworm Rearing Techniques in the Tropics*. Overseas technical co-operation agency: Tokyo, 180–199.
- Chinnaswami, K. P. (1983) Studies on Aspergillosis of silkworm, *Bombyx mori* L. caused by *Aspergillus tamarii* Kita. *M.Sc. (Ag.) Thesis*, University of Agricultural Sciences, Bangalore, 98.
- Finney, D. J. (1971) *Probit analysis—a statistical treatment of the sigmoid response curve*, 2nd edn. University Press: Cambridge.

- Govindan, R. and Devaiah, M. C. (1995) Bacterial flacherie of silkworm. *Silkworm Pathology Technical Bulletin* 3: 1–169.
- Govindan, R., Narayanaswamy, T. K. and Devaiah, M. C. (1998) *Principles of Silkworm Pathology*, SERI Scientific Publishers: Bangalore, 31–33.
- Jayaramaiah, M. (1981) Studies on the entomogenous fungus, *Beauveria brongniartii* (Sacc) Petch. in relation to white grub, *Holotrichia serata* Fab. and silkworm, *Bombyx mori* L. and possibilities of its use in the management of the white grub, *Ph.D. Thesis*, University of Agricultural Sciences, Bangalore, p. 246.
- Mallikarjuna, B. (1998) Studies on the effect of systematic fungicide on pathological changes in silkworms, infected with *Beauveria bassiana*, *M.Sc. Dissertation*, University of Mysore, Mysore, 1–5.
- Jhansi Lakshmi, V. V. N. S. (2003) Ultrastructural studies on tissues of the silkworm, *Bombyx mori* L. infected with *Beauveria bassiana* (Balsamo) Vuillemin, *Ph.D. Thesis*, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, 1–16.
- Krishnaswamy, S. (1978) New Technology of silkworm rearing. *C.S.R. and T.I. Bulletin* 2: 1–23.
- Krishnaswamy, S. (1979) Improved method of rearing youngage silkworms. *CSR and TI Bulletin* 3: 1–24.
- Paillot, A. (1930) *Traits des maladies du vera soiet Doin et Cie, Paris*, 279.
- Raghavaiah, G. and Jayaramaiah, M. (1989) Lethal concentration of white muscardine fungus, *Beauveria bassiana* (Bals.) Vuill. to different races of mulberry silkworm. *Indian Journal of Sericulture* 28(1): 94–96.
- Raghavaiah, G. and Jayaramaiah, M. (1990) Susceptibility of some races of the silkworm, *Bombyx mori* to white muscardine disease. *Indian Journal Sericulture* 29(2): 304–307.
- Reddy, M. V. R. (1978) Studies on white muscardine disease of silkworm, *Bombyx mori* L. *M.Sc. (Ag.) Thesis*, University of Agricultural Sciences, Hebbal, Bangalore, 92.
- Steinhaus, E. A. (1949) *Principles of the insect pathology*, McGraw Hill Book Co. Inc.: New York, 57.
- Suryanarayana Murty, U., Sai, K. S. K., Satya Kumar, D. V. R., Sriram, K., Madhusudhan Rao, K., Krishna, D. and Murty, B. S. N. (2002) *Relative abundance of Culex quinquefasciatus (Diptera: Culicidae) with reference to infection and infectivity rate from rural and urban areas of East and West Godavari districts of Andhra Pradesh, India*, Southeast Asian Tropical Medicine and Public Health: (In Press)
- Werren, J. H. (1997) Wolbachia run a mok. *Proceedings of National Academy of Sciences, USA* 94: 11154–11155.

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## Potential for control of the oriental latrine fly, *Chrysomya megacephala* in south-west India using insecticide-treated targets

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**ABSTRACT:** A control trial was carried out at a fish processing site, Puthyiappa, near Calicut in south-west India against *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) using cloth targets, which had been dipped in 7.5% deltamethrin suspension. The population at a second fish processing site, Vellayil, 6 km south of Puthyiappa, was also monitored during this period. Treatment resulted in a significant local reduction in *C. megacephala* population, but this effect was relatively limited in space and degree. The reasons for the apparently limited efficacy and the potential for use of such technology for *C. megacephala* control are discussed.

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**KEYWORDS:** *Chrysomya megacephala*, control, insecticide-treated targets, India

### INTRODUCTION

The Oriental latrine fly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae), is an abundant and widely distributed species, found throughout the oriental and Australasian regions of the Palaearctic. In recent decades, it has expanded its range to Africa, South and North America (Prins, 1979; Olsen and Sidebottom, 1990; Wells, 1991; Wells and Greenberg, 1992). It is normally saprophagous (Wijesundara, 1957), acting only occasionally as a secondary invader of myiases, often in association with the old-world screwworm fly, *Chrysomya bezziana* Villeneuve (Diptera: Calliphoridae) (Roy and Dasgupta, 1975). As a result of its abundance and synanthropic habit, it is an important vector of enteric pathogens, protozoans and helminths and has been associated with the transmission of Polio virus, *Escherichia coli*, *Giardia*, *Entamoeba coli* and *E. histolytica* (Greenberg, 1988). *C. megacephala*, is abundant throughout

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India, in both rural and urban habitats (White *et al.*, 1940; Roy and Dasgupta, 1975). In coastal regions of India in particular, infestation by *C. megacephala* is an important problem at fish landing and processing sites. On an average, 10% of sun-dried and stored dried fish in India is lost through infestation, largely by *C. megacephala* (Ward *et al.*, 1998). At a local scale, losses may be considerably greater, and may exceed 90%, particularly during and immediately following the monsoon (Golob *et al.*, 1987). These levels of infestation can result in extremely high fly population densities increasing the potential for human disease transmission too (Emerson *et al.*, 1999).

Traditionally processed, sun-dried fish is highly susceptible to attack by insect pests. Simple measures, such as raised tables aid drying and can help reduce fish infestation by fly larvae (Popham, 1980). Physically screening fish during the initial drying period may also be used to control infestation (Esser, 1991). Nevertheless, screening may often be ineffectual, as flies invariably find a gap in the screen to gain access to the fish, or may drop their eggs through the mesh of the screen (Walker and Wood, 1985). As a result, screens are often not considered economically viable or used widely by processors. Improving the natural drying process, using either natural convection dryers or solar dryers offers another alternative control technique (Nair *et al.*, 1994). However, these technologies are primarily of value when used on a small scale; studies suggest that they are unlikely to be of commercial use (Walker and Wood, 1985).

The control of larval infestation through the application of insecticides to fish during drying and storage can be a highly effective control strategy (Walker, 1987). Pyrethrins, usually synergised with piperonyl butoxide and pirimiphosmethyl are the only insecticides to have been allocated maximum permissible residue limits for fish by the FAO/WHO Joint Meeting on Pesticide Residues (JMPR). The maximum residue limit (MRL) was set at 3 mg/kg pyrethrin and 20 mg/kg piperonyl butoxide by WHO in 1970. A further advantage of synergised pyrethrins is their repellency, which discourages blowflies from landing and contaminating the fish. Synergised pyrethrins have usually been applied at a concentration of 0.3% (w/v) or less and some early studies reported good fly control in dried fish (McLellan, 1963; Green, 1967; Morris and Andrews, 1968). However, in 1978 Meynell (in FAO, 1981) reported that synergised pyrethrin did not give effective control of blowflies on sun drying fish unless it was applied at much higher dosages, which then created residues in excess of the MRL. Similar results have been reported widely in other studies (FAO, 1981; Walker and Donegan, 1984). Little information has been published on residues from treated fish. Nevertheless, synergised pyrethrins continue to be used by fish processors in developing countries. The organophosphate pirimiphosmethyl was shown to prevent infestation of fish in Malawi (Walker and Donegan, 1984) and Indonesia (Esser and Hanson, 1990). A MRL of 10 mg/kg has been recommended. Nevertheless, residues in excess of the MRL may occur; pirimiphosmethyl residues of over 26 mg/kg were recorded in Zambia in commercial dried fish (Walker, 1987). Hence, although these insecticides may be effective when applied

directly to the fish and safe when used correctly, they do not represent an ideal long-term remedy because they are often expensive, residues may cause harm to the consumer and environment and, with frequent use, may produce resistant strains of the pest. There is therefore a need to devise an inexpensive but effective means of reducing insect infestations, which can be used either independently or in conjunction with other control techniques currently available. In all fisheries there is considerable scope for increased hygiene and sanitation to reduce resident insect populations (Kordyl, 1976; Walker and Wood, 1985). Use of insecticides either broadcast into the environment or applied to the fish, remains common (Walker, 1987). In addition to the cost of extensive insecticide use, the application of insecticides to drying fish may also have unacceptable environmental and health consequences. Hence, the identification of alternative effective techniques for the control of *C. megacephala* would be of considerable value. The study reported here sought to develop a simple target, impregnated with the insecticide deltamethrin, which would attract and kill *C. megacephala*. Deltamethrin was selected because of its known efficacy against Diptera, availability and low cost (Torr *et al.*, 1992). A suspension concentrate formulation designed for use on targets for tsetse control was used. The deltamethrin-impregnated cloth targets showed no loss of insecticidal activity in the field over a five month period, which included the monsoon. This technique has been used effectively to control a range of insect pests, such as tsetse flies in sub-Saharan Africa (Torr *et al.*, 1992) houseflies and blowflies (Howard and Wall, 1996; Smith and Wall, 1998).

## MATERIALS AND METHODS

### Effects of deltamethrin-impregnated targets

In preliminary laboratory trials, the toxicity of deltamethrin to a representative blowfly species, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) was assessed. A polycotton cloth target (15 × 5 cm) dipped in 20 ml of 5 and 10% deltamethrin suspension was dried for two hours in a fume cupboard. The strip was hung in the centre of a mesh cage (30 × 30 × 30 cm), which contained granulated sucrose and water. The cage was then placed in an illuminated room. Forty five, six-day-old, non-protein-fed female *L. sericata* were released into each cage. Mortality (including insects knocked down) was recorded at 1.5, 3.5, 20.5 and 25.5 h after exposure. As a control, the mortality of *L. sericata* exposed in target dipped in sugar-solution alone was assessed.

### The persistence of deltamethrin on impregnated targets under field condition

Polycotton targets (50 × 15 cm) dipped in a 7.5% aqueous suspension of deltamethrin were hung outside, subjecting them to weathering in India. Every month during the course of the field trials reported below, one target was removed and used in a laboratory toxicity bioassay against *C. megacephala*. For this bioassay, strips, 15 × 5 cm were cut from the target and tested as described above.

### Field site and routine fly sampling

All field trials were carried out at the Puthiyappa in Kerala state in south-west India. On the site there are 100 sheds,  $5 \times 10$  m in area used for the storage of fish in various stages of processing. The key fish species handled are ribbonfish (*Trichiurus lepturus*), anchovy (*Stolephorous* spp.), sole (*Cynoglossus semifasciatus*), Indian mackerel (*Rastrelliger kanagurta*), lesser sardine (*Sardinella gibbosa*) and silverbelly (*Leiognathus bindus*). The peak period of fishing and fish processing occurs between August and November. Fish is dried on the sand, coir mats or on concrete drying areas and stored inside the sheds overnight in baskets or are sealed in polythene bags for onward sale.

Eight sheds belonging to one processor were selected for monitoring. To monitor the adult fly population, white targets ( $15 \times 20$  cm) were constructed of corrugated, plastic-coated card (Correx<sup>®</sup>). The targets were covered on one side with white, sticky fly paper (AgriSense BCS Ltd., Pontypridd), which was attached at the top with staples. Two targets were suspended from the eight sheds; one inside the shed from the roof and the other outside, from the eaves. Targets were deployed in April 1999 and remained in place until July 1999. Throughout this period flies were picked off the sticky targets at 3–4 d intervals, placed in labelled pots containing a petroleum-based solvent and taken back to the laboratory. The sticky paper was replaced if the surface was cluttered with fly debris or other material. In the laboratory, each pot was sieved and the flies allowed to dry on filter paper. All flies were then identified using a binocular, dissecting microscope.

### Control trial

Targets were made from white polycotton cloth strips ( $50 \times 15$  cm). The cloth strips were dipped in a 7.5% aqueous solution of deltamethrin suspension and dried for 24 h prior to use. In constructing the target, the bottom 10 cm of the cloth strip was folded-up to make a pocket, leaving a  $40 \times 15$  cm target. A frame of black corrugated plastic (Corex<sup>®</sup>) 4 mm in width, supported the four edges of the target. The target was suspended using a length of wire, attached to its top edge. In the field, 15 ml of butyric acid, heat-sealed in a  $10 \times 5$  cm plastic sachet of wall thickness 0.7 mm (Lay-flat tubing, Transatlantic Plastics Ltd., Southampton) was placed into the pocket at the base of each target. Previous work has shown that visual stimuli and, in particular, colour-contrast is an important component in the design of targets which are attractive to Diptera (Hall, 1995; Howard and Wall, 1998). Hence, a contrasting black frame supporting a vertical white target was employed for the current control trials. The targets were also baited with butyric acid since this chemical has been shown to be attractive to blowflies (Fisher, 1999) and is widely used in a range of baits such as swormlure for the screwworm *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) (Mackley and Brown, 1984) and lucilure for the blowfly *Lucilia cuprina* (Weidemann) (Diptera: Calliphoridae) (Hall, 1995).

A total of 398 insecticide-treated targets were deployed at the Puthiyappa site. Targets were deployed in April, when the fly population was believed to be at its

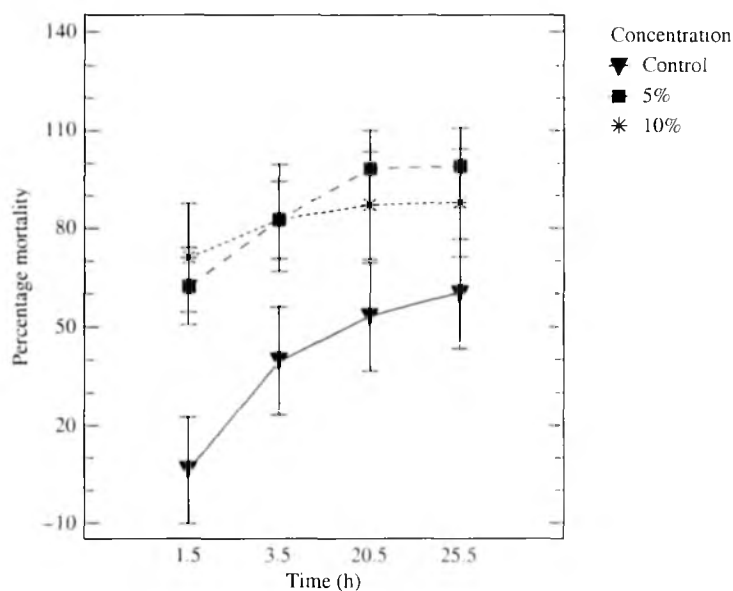


FIGURE 1. The mean percentage mortality (knockdown)  $\pm 95\%$  confidence interval, of adult *Lucilia sericata* after exposure in the laboratory to a polycotton cloth target ( $15 \times 5$  cm), which has been dipped in 5 or 10% deltamethrin suspension concentrate or an untreated target.

seasonal minimum. Targets were placed under the eaves of every house or processing shed as the site. Most sheds received 4 insecticide-treated targets and the largest sheds up to 9 targets.

During the control trial, the fly population at a second fish processing site at Vellayil, 6 km south of Puthiyappa, was also monitored using ten sticky targets and population was assessed following the method described earlier. Data were subjected to analysis of variance.

## RESULTS

### Effects of deltamethrin-impregnated targets

In the laboratory trials with *L. sericata*, deltamethrin gave almost 100% kill in 2 hours (Fig. 1). There was no significant difference in the efficacy of the two doses used.

### Persistence of deltamethrin on impregnated targets exposed in the field

The two-month old target showed the highest fly mortality against *C. megacephala*, while the one, three and four month-old targets were of lower but equivalent toxicity (Fig. 2).

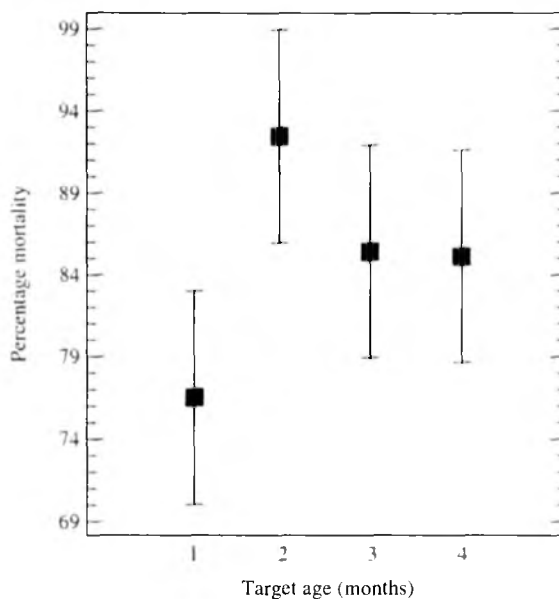


FIGURE 2. The percentage mortality (knockdown)  $\pm 95\%$  confidence interval, of adult *Chrysomya megacephala* after exposure for 24 h to a polycotton cloth target in the laboratory, which had been dipped in deltamethrin suspension concentrate (7.5%) and then exposed to weathering in the field in India for 1, 2, 3 or 4 months, between April and August 1999.

### Control trial

The deltamethrin-impregnated targets were lost from the Puthiyappa site in Calicut relatively rapidly. This was probably the result of monsoon storms. The targets remaining out of the 398 insecticide-impregnated targets initially placed at the end of each month were as follows. May (357), June (240), July (168), August (163), and September (148), with the sampling sheds, numbered 1 to 8 from the beach to the road, possessing 0, 1, 0, 2, 3, 4, 4 and 3 insecticide-impregnated targets respectively.

The mean catches of *C. megacephala* were low throughout May but they increased rapidly with the onset on the monsoon rains in June (Fig. 3). Fly abundance then passed through a series of peaks and troughs at both sites. Overall, there was no significant difference in the abundance of *C. megacephala* at the Puthiyappa and Vellayil sites. However, at the Puthiyappa site the catches in sheds 1 and 8 were relatively high compared to sheds 2 to 7 (Fig. 4). Both sheds 1 and 8 were at the periphery of the control area and, therefore, were most likely to be affected by immigration. In addition, as described above, shed 1 was unprotected by insecticide-impregnated targets for most of the trial period. If shed 1 is removed from the sample, analysis of variance in which the  $\log_{10}$  number (+1) of *C. megacephala* is the dependent variable and site and shed number are treated as factors, the population

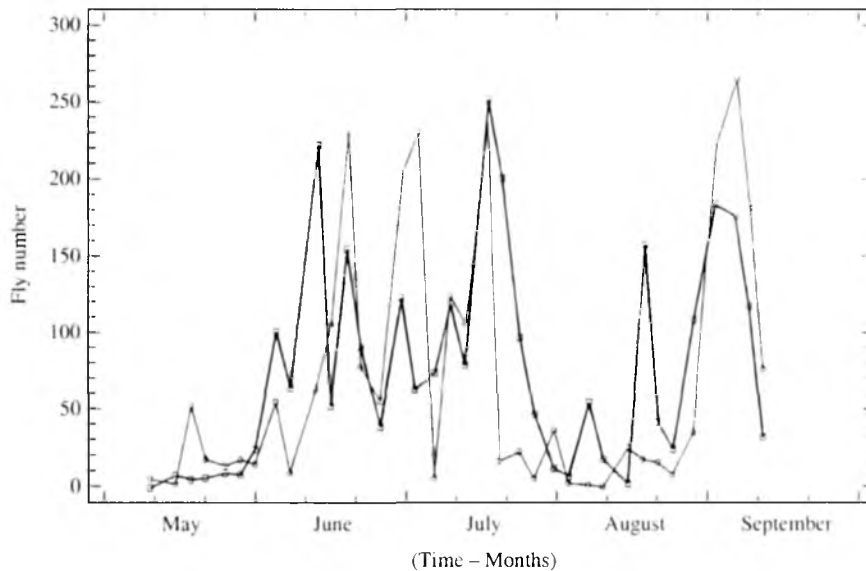


FIGURE 3. Mean number of *Chrysomya megacephala* caught per sampling shed between April and September 1999 (day 1 = 23rd of April) at the Puthyiappa site (square symbols, thick line) and the Vellayil site (circles, thin line) in southwest India.

of *C. megacephala* at the Puthyiappa is shown to be significantly lower than that at the Vellayil site ( $F_{1,1727} = 3.9$ ,  $P < 0.05$ ).

#### DISCUSSION

At the Puthyiappa site in south-west India, *C. megacephala* is the dominant fly species, accounting for over 95% of the adult flies caught and 70% of flies infesting the drying fish (Wall *et al.*, 2001). The populations of *C. megacephala* show strong seasonal patterns of abundance, declining at the end of the monsoon and remaining low during the dry period, before rising again rapidly at the start of the monsoon rains in June. Despite the deployment of 398 cloth targets treated with 7.5% deltamethrin, there was no significant overall difference between the abundance of *C. megacephala* at the Puthyiappa site and the Vellayil site, where no insecticide-treated targets were present. Nevertheless, closer inspection of relative *C. megacephala* abundance on a shed by shed basis suggested that there might have been some significant local reduction in abundance at the centre of the control site. Across the Puthyiappa site, the catches in the two sheds at the periphery of the control area were relatively high compared to sheds positioned more centrally within the site. These two sampling sheds are the most likely to have been affected by immigration. Furthermore, the shed by the beach (shed 1) had no insecticide-impregnated targets remaining on it by July. Hence it is perhaps unsurprising that a locally high *C. megacephala* population was recorded at

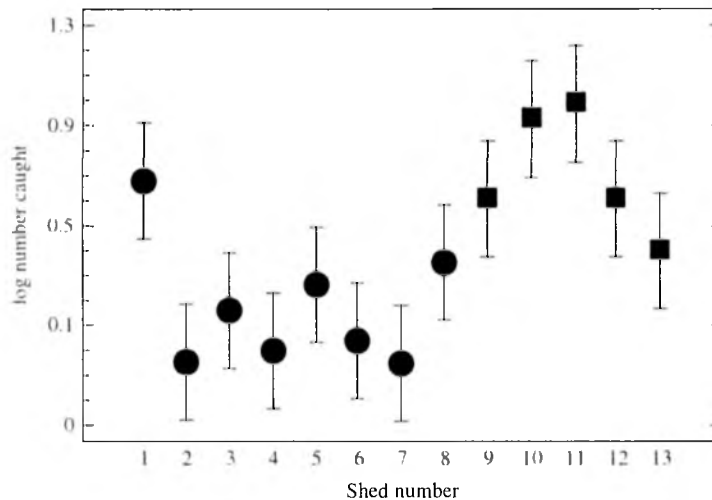


FIGURE 4. The mean  $\log_{10}$  number (+1) of adult *Chrysomya megacephala*,  $\pm 95\%$  confidence interval, caught between 23rd of April 1999 (day 1) and August 1999, in eight fish storage sheds at the Puthiyappa site (numbers 1 to 8, circled) and five sheds at the Vellayil site (numbers 9 to 13, squares) in southwest India.

this shed. If shed 1 is excluded from the subsequent analysis the population of *C. megacephala* recorded on sheds 2–7, within the Puthiyappa site, were significantly lower than that at the Vellayil site over the same time period.

The use of the Vellayil site as a control for this study is problematical. In reality, with such variable and complex systems true controls are difficult to identify. Villages cannot be replicated exactly in space and time. And, as is to be expected, the fish processing methods at the Puthiyappa and Vellayil sites did differ to some degree. At Vellayil the main products are wet, gutted pelagic fish, such as sardines and mackerel, with some but less sun drying than at Puthiyappa. Since the Vellayil processors do not sun dry as much fish as those at Puthiyappa there is less open ground in the area and salting sheds are closer together. Generally the processors at Puthiyappa operate at a larger scale than those in Vellayil. Such differences were unavoidable in the selection of any control site. It could be argued that to make comparisons between the Puthiyappa and Vellayil sites more valid, baseline monitoring should have been carried out the previous year. However, the previous year's fly catch from either Puthiyappa or Vellayil would provide relatively little useful information since fly abundances can change dramatically from year to year depending on climatic conditions.

The results suggested that the cloth dipped in deltamethrin was least toxic, one month after exposure to sunlight and most toxic after two month exposure. This result is surprising given that exposure to sunlight and rain would have been expected to reduce the toxicity of the deltamethrin. Further work would be required to confirm this observation. Nevertheless, the targets dipped in 7.5% deltamethrin remained highly

toxic to *C. megacephala* and other blowflies in laboratory bioassays throughout the trial. The conclusion of this study, however, is that although targets remained toxic, the presence of the targets created little reduction in *C. megacephala* abundance, and then only at the centre of the Puthiyappa site.

There are a number of possible reasons for this result. The first is that following deployment in April 1999, the number of targets present at the field site declined relatively quickly. Some were probably blown away in the monsoon storms. A census carried out at the end of July, half-way through the trial, showed that only 168 targets remained. This may have been too few to produce any substantive effect on the *C. megacephala* population. The second, and equally significant reason is likely to have been the fact that the control site could not be isolated and high level of immigration occurred. To an extent this immigration pressure can be inferred from the pattern of trap-catches at the Puthiyappa site shown in Fig. 4. Clearly, the use of target-technologies, while showing promise for the control of many insect pest species, would need to be used on an area wide scale with the active co-operation and support of local communities, to be effective against such widely dispersed and locally abundant species as *C. megacephala*.

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- Emerson, P. M., Lindsay, S. W., Walraven, E. L., Faal, H., Bogh, C., Lowe, K. and Bailey, R. L. (1999) Effect of fly control on trachoma and diarrhoea. *The Lancet* **353**: 1401–1403.
- Esser, J. R. (1991) Biology of *Chrysomya megacephala* (Diptera: Calliphoridae) and reduction of losses caused to the salted-dried fish industry in south-east Asia. *Bulletin of Entomological Research* **81**: 33–41.
- Esser, J. R. and Hanson, S. W. (1990) Prevention of insect infestation and losses in salted-dried fish in Indonesia by treatment with an insecticide approved for use on fish. *Indopacific Fishery Commission*. In: Paper presented at the seventh session of the Indo-Pacific Fishery Commission Working Party on Fish Technology and Marketing Bangkok (Thailand, 19–22 April). 308–FAO Fisheries Report, No. 401 (Suppl.) FAO, Rome.
- FAO, (1981) The prevention of losses in cured fish. In: *FAO Fisheries. Technical. Paper*, FAO: Rome, vol. **219**: 87.
- Fisher, P. (1999) Responses of the blowfly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) to semiochemical baits. *Ph.D. Thesis*, University of Bristol.
- Golob, P., Cox, J. R. and Kilminster, K. (1987) Evaluation of insecticide dips as protectants of stored dried fish from dermestid beetle infestation. *Journal of Stored Products Research* **23**: 47–56.
- Green, A. A. (1967) The protection of dried sea-fish in south Arabia from infestation by *Dermestes frishii* Kug. (Coleoptera: Dermestidae). *Journal of stored Products Research* **2**: 331–350.

- Greenberg, B. (1988) *Chrysomya megacephala* (F) (Diptera: Calliphoridae) collected in North America and notes on *Chrysomya* species present in the New World. *Journal of Medical Entomology* **25**: 199–200.
- Hall, M. J. R. (1995) Trapping the flies that cause myiasis: their responses to host stimuli. *Annals of Tropical Medicine and Parasitology* **89**: 333–357.
- Howard, J. J. and Wall, R. (1996) Autosterilization of the housefly, *Musca domestica*, in poultry houses in northern India. *Bulletin of Entomological Research* **86**: 363–367.
- Howard, J. J. and Wall, R. (1998) The effects of contrast on the attraction of the house fly, *Musca domestica*, to visual targets. *Medical and Veterinary Entomology* **12**: 322–324.
- Kordyl, E. (1976) Some protective measures against insect infestation of dried fish in Africa. In: *Proc. of the Conference on Handling, Processing and Marketing of Tropical Fish*: London, 313–314.
- Mackley, J. W. and Brown, H. E. (1984) Swormlure-4: A new formulation of the Swormlure-2 mixture as an attractant for adult screwworms *Cochliomyia hominivorax* (Diptera: Calliphoridae). *Journal of Economic Entomology* **77**: 1264–1268.
- McLellan, R. H. (1963) The use of pyrethrum dips as protection for drying fish in Uganda. *Pyrethrum Post* **7**: 8–10.
- Morris, R. F. and Andrews, D. (1968) Investigations into the use of pyrethrum and other insecticides for the control of the blowfly *Calliphora terraenovae* (Macq.), infesting ligh-salted cod in Newfoundland. *Pyrethrum Post* **9**: 9–12.
- Olsen, A. R. and Sidebottom, T. H. (1990) Biological observations on *Chrysomya megacephala* (Fabr.) (Diptera: Calliphoridae) in Los-Angeles, California and the Palau-Islands. *Pan-Pacific Entomologist* **66**: 126–130.
- Popham, E. J. (1980) Inexpensive means of controlling insect infestations of dried fish from Lake Chilwa, Malawi. *Luso Journal of Science and Technology (Malawi)* **1**: 55–61.
- Prins, A. J. (1979) Discovery of the oriental latrine fly *Chrysomya megacephala* (Fabricius) along the south-western coast of South Africa. *Annals of the South African Museum* **78**: 39–47.
- Ravindranathan Nair, P., Unnikrishnan Nair, T. S., Mathen, C. and Joseph, K. G. (1994) Thermal treatment for the prevention of Insect infestation in dried fish—use of a tunnel drier/solar tent drier. *Fishery Technology* **31**: 133–141.
- Roy, P. and Dasgupta, B. (1975) Seasonal occurrence of muscid, calliphorid and sarcophagid flies in Silguri, West Bengal, with a note on the identity of *Musca domestica* L. *Oriental Insects* **9**: 351–374.
- Smith, K. E. and Wall, R. (1998) Suppression of a population of the blowfly *Lucilia sericata* in sheep pastures using baited targets. *Medical and Veterinary Entomology* **12**: 101–108.
- Torr, S. J., Holloway, M. T. P. and Vale, G. A. (1992) Improved persistence of insecticide deposits on targets for controlling *Glossina pallidipes* (Diptera: Glossinidae). *Bulletin of Entomological Research* **82**: 525–533.
- Walker, D. J. (1987) A review of the use of contact insecticides to control post-harvest insect infestation of fish and fish products. *FAO Fisheries Circular* 804:19: FAO, Rome.
- Walker, D. J. and Donegan, L. (1984) Spray application of insecticides to protect sundrying fish from blowfly infestation during the wet season in Malawi. *International Pest Control* **5**: 132–135.
- Walker, D. J. and Wood, C. D. (1985) Non-insecticidal methods of reducing losses caused by infestation of blowflies (Diptera) during fish curing procedures. In: *Proc. of the FAO Expert Consultation on Fish Processing in Africa*. FAO Fisheries Report No. 329(Suppl.) Rome.
- Wall, R., Howard, J. J. and Bindu, J. (2001) The seasonal abundance of flies infesting drying fish in southwest India. *Journal of Applied Ecology* **38**: 339–348.
- Ward, A. R., Schoen, V., Joseph, M. J., Kumar, S. and Cunah, J. D. (1998) Monsoon post harvest fish losses in India. In: *Advances and Priorities in Fisheries Technology*, Balachandran, K. K., Iyer, T. S. G., Madhavan, P., Joseph, J., Perigreen, P. A., Raghunath, M. R. and Varghese,

- M. D. (Eds). Cochin, India (pp. 478–483).
- Wells, J. D. (1991) *Chrysomya megacephala* (Diptera: Calliphoridae) has reached the continental United States: review of its biology, pest status, and spread around the world. *Journal of Medical Entomology* **28**: 471–473.
- Wells, J. D. and Greenberg, B. (1992) Interaction between *Chrysomya rufifacies* and *Cochliomyia macellaria* (Diptera: Calliphoridae): the possible consequences of an invasion. *Bulletin of Entomological Research* **82**: 133–137.
- White, R. S., Aubertin, D. and Smart, J. (1940) *The Fauna of British India. In: Diptera*. Vol VI. Family Calliphoridae, *Taylor and Francis Ltd: London*.
- Wijesundara, D. P. (1957) The life-history and bionomics of *Chrysomya megacephala* (Fab.). *Ceylon Journal of Science* **25**: 169–185.

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## New record of whiteflies (Aleyrodidae: Hemiptera: Insecta) in Mangrove forests of Southern India

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**ABSTRACT:** Whiteflies colonizing on the mangrove plant *Excoecaria agallocha* have been reported for the first time from India. Five species of whiteflies viz., *Aleurocanthus martini* David, *Aleurocanthus rugosa* Singh, *Aleuroclava* sp. indet., *Aleurolobus marlatti* (Quaintance) and *Aleuroplatus alcocki* (Peal) have been noticed to occur on the mangrove in Andhra Pradesh whereas only *Aleuroplatus alcocki* (Peal) was noticed in Tamil Nadu. A field identification key is provided for easy identification of mangrove whiteflies. © 2005 Association for Advancement of Entomology

**KEYWORDS:** Mangroves, *Excoecaria agallocha*, Pichavaram, mangrove whiteflies.

### INTRODUCTION

Mangrove forest represents a highly specialized and complex ecosystem occurring in sheltered coastal areas of tropics and subtropics. Among the many ecosystems, the mangrove forest is unique due to the edaphic and climatic factors as well as the typical flora. The rich floral diversity of mangrove forests harbour many varieties of insects viz., mostly coccids, mosquitoes, biting midges, beetles, termites, ants, honey bees, bugs and borers (Saxena, 1994). Since there has been no report of occurrence of whiteflies on mangroves, the present survey was undertaken to study the aleyrodid fauna of mangrove forest in the south east coast of India.

### MATERIALS AND METHODS

Intensive survey was carried out during February and March 2003 in the Pichavaram mangroves located between 11° 27' N and 79° 47' E in the Cuddalore District of Tamil

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Nadu and Godavari Mangroves located between 160 30'–170 N' and 820 10'–820 23'E in the East Godavari District of Andhra Pradesh of the south east coast of India. The whitefly infested leaves of mangroves were collected in specially designed covers and mounts were prepared from the adult emerged and unparasitised puparia.

## RESULTS

### *Taxonomy*

1. *Aleurocanthus martini* David (Fig. 1).

*Aleurocanthus martini* David 1993. *FIPAT Entomology Series*, 3: 12.

This species was first reported from Sri Lanka on *Sebastiana chamelia* (David, 1993). Sundararaj and Dubey (2003) redescribed it in detail.

### *Distribution*

India–Karnataka: Bangalore (Sundararaj and Dubey, 2003); Andhra Pradesh: Kakinada (new distribution record).

### *Host Plants*

*Santalum album* (Sundararaj and Dubey, 2003); *Excoecaria agallocha* (new host record).

### *Material examined*

India-Andhra Pradesh : Kakinada, on *Excoecaria agallocha*, 16.iii.2003, E. Ragupathy, 6 puparia on slides.

2. *Aleurocanthus rugosa* Singh (Fig. 2)

*Aleurocanthus rugosa* Singh, 1931. Mem. Dep. Agric. India, 12(1): 71.

*Aleurocanthus rugosa* David and Subramaniam, 1976. *Rec. Zool. Surv. India*, 70: 151.

### *Distribution*

India-Bihar (Pusa) (Singh, 1931); India-Tamil Nadu (Coimbatore, Madras) (David and Subramaniam, 1976), (Madras) (Jesudasan and David, 1991); India-Andhra Pradesh, Kakinada (new distribution record).

### *Host Plants*

*Syzgium jambolanum*, *Piper betel*, *Psidium guajava*, *Michelia champaca* (Singh, 1931); *Annona* sp., *Polyalthia longifolia*, *P. pendula* (David and Subramaniam, 1976); *Dodonaea viscosa* (Jesudasan and David, 1991); *Excoecaria agallocha* (new host record).

**Material examined**

India-Andhra Pradesh: Kakinada, on *Excoecaria agallocha*, 16.iii.2003, E. Ragupathy, 14 puparia on slides.

3. *Aleuroclava* sp. indet.

During the survey only a single parasitised specimen was collected.

*Distribution*

India- Andhra Pradesh: Kakinada (new distribution record).

*Host Plant*

*Excoecaria agallocha* (new host record).

*Material examined*

India-Andhra Pradesh : Kakinada, on *Excoecaria agallocha*, 16.iii.2003, E. Ragupathy, 1 puparium on slide, parasitised.

4. *Aleurolobus marlatti* (Quaintance) (Fig. 3)

*Aleurolobus niloticus* Priesner and Hosny, 1934. *Bull. Minist. Agric. Egypt. Tech. Scient. Serve.*, **145**: 1–5. [Synonymised by Martin, 1999].

*Aleurolobus ravisei* Cohic, 1968. *Cah. Off. Rech. Sci. Tech. Outre-Mer. (Biologie)*, **6**: 95–98.

*Aleurolobus niloticus* Priesner & Hosny. Cohic, 1969. *Annls. Univ. Abidjan (E)*, **2**: 50.

*Aleurolobus niloticus* Priesner & Hosny. Habib & Farag, 1970. *Bull. Soc. Entomol. Egypte*, **54**: 21.

*Aleurolobus niloticus* Priesner & Hosny. Hayat, 1972. *Entomophaga*, **17**: 100.

*Aleurolobus niloticus* Priesner & Hosny. Bink-Moenen, 1983. *Monografieen Van de Nederlandse Entomologische Vereniging*, **10**: 50–52.

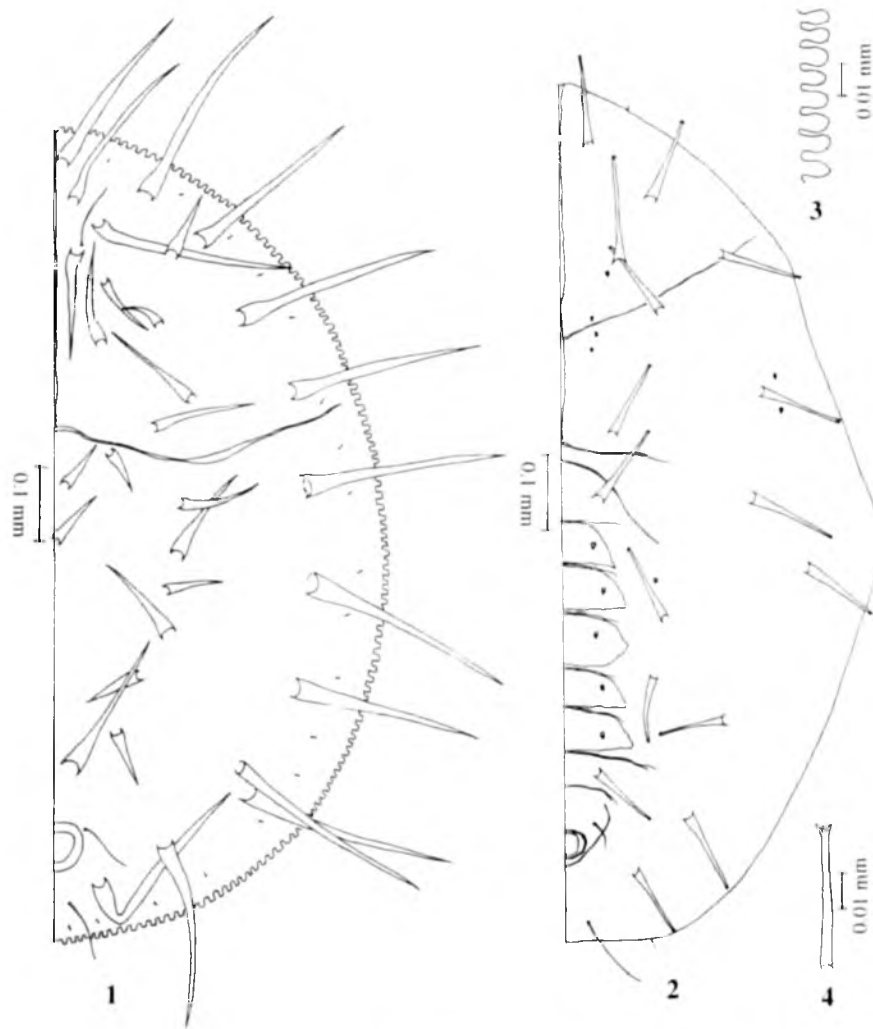
*Aleurolobus niloticus* Priesner and Hosny, 1934. Regu and David, 1993, *FIPPAT Entomology Series*, **4**: 33–34.

*Distribution*

This is a cosmopolitan species widely distributed throughout the country.

*Host plants*

A wide range of host plants includes 40 plant species belonging to 23 families including several weeds, ornamentals, mulberry and forest trees (Regu and David, 1993); *Excoecaria agallocha* (new host record).



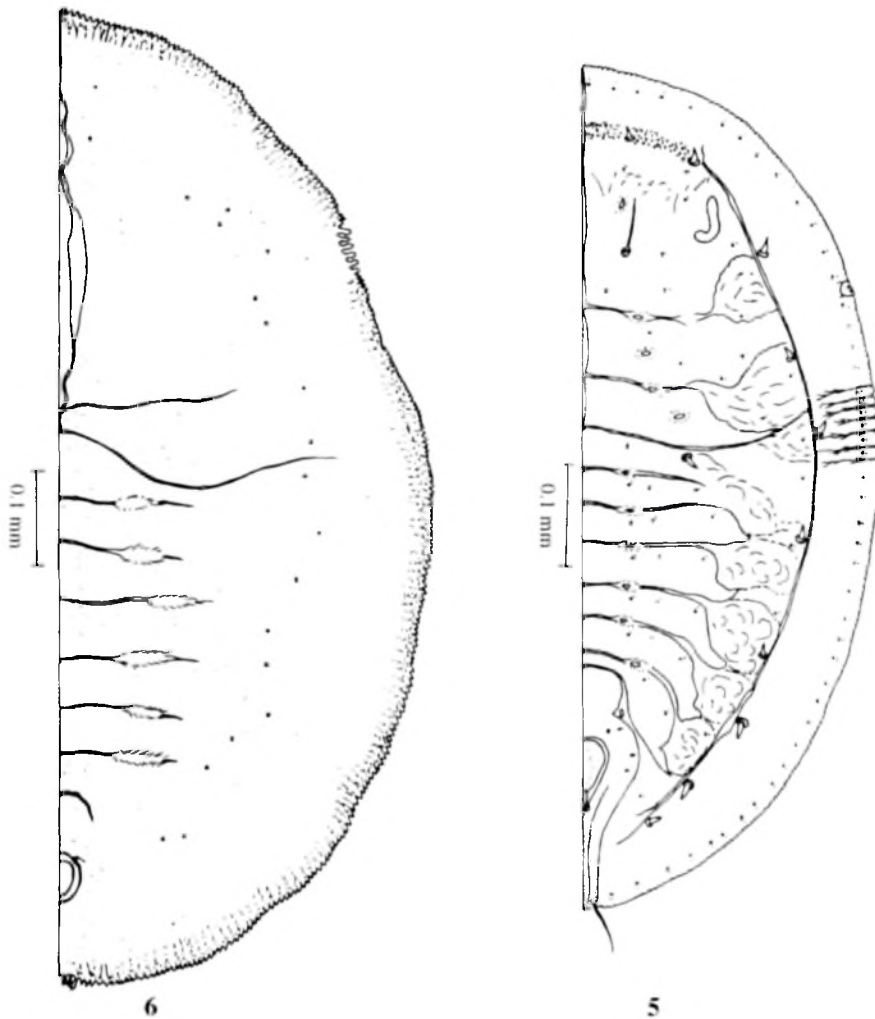
FIGURES 1–4: 1. *Aleurocanthus martini* David Puparium; 2. *Aleurocanthus rugosa* Singh, Puparium. 3. Margin, 4. Fimbriate spine.

#### *Material examined*

India-Andhra Pradesh,: Kakinada, on *Excoecaria agallocha*, 16.iii.2003, E. Ragupathy, 2 puparia on slides.

#### 5. *Aleuroplatus alcocki* (Peal) (Fig. 4)

*Aleurodes alcocki* Peal, 1903. *J. Asiatic Soc. Bengal*, **72**(2): 61–98.



FIGURES 5-6: 5. *Aleurolobus marlatti* (Quaintance), Puparium; 6. *Aleuroplatus alcocki* (Peal), Puparium.

*Aleuroplatus alcocki* Quaintance and Baker, 1914. U.S.D.A. *Bur. Ent. Tech. Ser.*, **27**:98

*Aleuroplatus pectiniferus* Quaintance and Baker, 1917. *Proc. U. S. Nat. Mus.*, **51**: 335-445.

*Aleuroplatus ficusgibbosae* Corbett, 1926. *Bull. Ent. Res.*, **16**: 267-284 (Synonymized by David, 1993).

*Aleuroplatus buchananianae* Jesudasan and David, 1991. *Oriental Insects*, **25**: 231-434 (Synonymised by Martin, 1999).

*Aleuroplatus cinnamomi* Jesudasan and David, 1991. *Oriental Insects*, **25**: 231–434 (Synonymised by Martin, 1999).

*Aleuroplatus distinctus* Jesudasan and David, 1991. *Oriental Insects*, **25**: 231–434 (Synonymized by Martin, 1999).

*Aleuroplatus pectenserratus* Singh: Martin, 1999. *CSIRO Entomology Technical Paper*, **38**: 48–49 (Synonymized by Martin, 1999).

*Aleuroplatus walayarensis* Jesudasan and David, 1991. *Oriental Insects*, **25**: 231–434 (Synonymized by Martin, 1999).

### *Distribution*

Widely distributed throughout India.

### **Host Plants**

A wide range of host plants (David and Subramaniam, 1976; Jesudasan and David, 1991). Recently, Jesudasan (2004) reported around 30 host plants including plantation crops, forest flora and weeds along the eastern and western ghats of India. *Excoecaria agallocha* is a new host.

### *Materials examined*

India–Tamil Nadu: Pichavaram, on *Excoecaria agallocha*, 29.ii.2003; E. Ragupathy, 12 puparia on slides; India–Andhra Pradesh: Kakinada, on *Excoecaria agallocha*, 16.iii.2003, E. Ragupathy, 10 puparia on slides.

## DISCUSSION

Russell (1963) first reported occurrence of *Trialeurodes vaporariorum* (Westwood) on *Avicennia nitida*. A perusal of Indian literature as well as the world catalogue of whiteflies (Mound and Halsey, 1978) has shown that occurrence of whiteflies on mangroves has not been reported so far from India. In the present study in Andhra Pradesh the mangrove plant *Excoecaria agallocha* has been found infested with five species of whiteflies viz., *Aleurocanthus martini* David, *Aleurocanthus rugosa* Singh, *Aleuroclava* sp. indet., *Aleuroplatus alcocki* (Peal) and *Aleurolobus marlatti* (Quaintance). Interestingly, only *A. alcocki* was observed on mangroves of Pichavaram, Tamil Nadu.

### *Field identification key*

1. Puparium white ..... 2
  - Puparium black ..... 3
2. Dorsal spines fimbriate; exuviae of the previous instar attached on the puparium *Aleurocanthus rugosa* (Singh)
  - Dorsal spines absent ..... *Aleuroclava* sp. indet.

3. Puparium embedded in the gummy wax . . . . . *Aleuroplatus alcocki* (Peal)  
– Puparium not embedded in the gummy wax . . . . . 4
4. Wax fringe covered around the puparium and dorsal spines pointed *Aleurocanthus martini* David  
– Wax fringe wanting; puparium without dorsal spine . . . . . *Aleurolobus marlatti* (Quaintance)

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#### REFERENCES

- David, B. V. (1993) The Whitefly of Sri Lanka. *FIPPAT Entomology Series* **3**: 12.
- David, B. V. and Subramaniam, T. R. (1976) Studies on some Indian Aleyrodidae. *Records of the Zoological Survey of India* **70**: 133–233.
- Jesudasan, R. W. A. (2004) Systematics of whiteflies in the Eastern and Western Ghats of India. In: *A technical report Submitted to DST*, New Delhi, 50.
- Jesudasan, R. W. and David, B. V. (1991) Taxonomic studies on Indian Aleyrodidae (Insecta: Homoptera). *Oriental Insects* **25**: 231–434.
- Martin, J. H. (1999) The whitefly fauna of Australia (Sternorhyncha: Aleyrodidae) A taxonomic account and identification guide. *CSIRO Entomology Technical Paper No* **38**: 1–197.
- Mound, L. A. and Halsey, S. H. (1978) *Whitefly of the world*, British Museum (Natural History), Wiley: Chichester, 340.
- Regu, K. and David, B. V. (1993) Taxonomic studies on Indian Aleyrodids of the Tribe Aleurolobini. *FIPPAT Entomology Series* **4**: 33–34.
- Russell, L. M. (1963) Host and distribution of five species of *Trialeurodes* (Homoptera : Aleyrodidae). *Annals of Entomological Society of America*. **56**: 149–153.
- Saxena, A. (1994) Fauna of Mangroves and its Management. In: *Conservation of Mangrove Forest Genetic Resources: A Training Manual*, Deshmukh, Sanjay V. and Balaji, V. (Eds). MSSRF: India, 240–253.
- Singh, K. (1931) A contribution towards our Knowledge of the Aleyrodidae (whiteflies) of India. *Memoirs of the Department of Agriculture in India* **12**(1): 1–98.
- Sundararaj, R. and Dubey, A. K. (2003) Whiteflies (Hemiptera: Aleyrodidae) associated with sandal (*Santalum album* L.) in southern India with description of a new species. *Entomon* **28**(4): 293–298.

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## Population size and composition in *Odontotermes brunneus* (Hagen) (Isoptera: Termitidae) in relation to mound size and seasons

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**ABSTRACT:** *O. brunneus* is a widely distributed mound building termite in Kerala. Mound density varied from 8–10/ha. The total population was directly proportional to the size of the mound and weight of the fungus garden. Mounds of *O. brunneus* varying in height from 2–27 cm and 11.9–115 cm in circumference at the base, contained a population ranging from 3900–70,700 individuals. A mound 14 cm high and 72 cm in circumference at the base has a population of  $27,042 \pm 20,644$  individuals, comprising 60.68% nymphs, 31.45% workers, 3.98% pre-soldiers and 3.89% soldiers. The population showed seasonal fluctuation. Mature alates were recorded during March, April, September and October. The proportion of workers inside the mound (8.75%–12.18%) was low during June–October. This is attributed to increased foraging during the rainy season. High percentage of larvae were found during June, July and August (79.04–81.6%) indicating peak egg production during the monsoon season. © 2005 Association for Advancement of Entomology

**KEYWORDS:** *O. brunneus*, population, seasonal fluctuation

### INTRODUCTION

Termites form a dominant group among soil dwelling organisms in all the warmer regions of the world. The total population, proportion of various castes and seasonal fluctuation have been studied in detail, especially in the family Termitidae (Sen-Sarma and Mishra, 1977; Darlington, 1990; Aktar and Rashid, 2001). In Kerala practically no work on the population of any species has been carried out so far, notwithstanding its rich termite fauna. *O. brunneus* is a widely distributed species found here, building dome shaped mounds (Miranda and Prabhoo, 1990). This study was carried out to determine the mound density, total population in mounds and seasonal population fluctuation.

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## MATERIALS AND METHODS

The study was carried out in about 1500 ha of the Kerala University Campus, Kariavattom, Trivandrum, Kerala in 1996. The climate is tropical, with three well marked rainy seasons in a year—pre-monsoon period (March–May), monsoon period (June–August) and post-monsoon period (October–November). The soil is primarily lateritic.

To determine the density of the mounds in the study site, ten plots of 1 ha each were selected at random and the mounds were directly counted from each plot.

To determine the population density, ten mounds varying in height and basal circumference were selected. Each one was quickly broken open and five samples of fungus garden (100 g each) from different parts of the mound at random were taken carefully and quickly before the termites could respond to the disturbance. Each sample was kept in a separate tray and the various castes such as workers, soldiers, pre-soldiers and larvae were separated by hand sorting. Individuals of each caste were counted from all the five samples and the mean value was determined. The entire fungus garden from the mound was collected and air-dried the same day of collection to prevent degradation. When the combs were dry and hard, they were broken up and cleaned with a dry paint brush to dislodge dead termites and soil particles. These were oven dried at 50 °C and weighed. The total population was calculated on the basis of the total weight of the fungus garden.

To study the seasonal fluctuations in the population, mature mounds of *O. brunneus* were chosen (maturity was determined solely on the production of a large brood of alates the previous year). Six mounds varying from 22–27 cm in height were selected. Population estimation per 100 g of fungus garden was made at monthly intervals for one year. By the principle of least squares, the relationship between the height/circumference of the mound ( $X$ ) and total population ( $Y$ ) can be established by the curve of the form  $Y = AB^X$  where  $A$  and  $B$  are population parameters estimated from the sample. Regression analysis to ascertain the relationship between the weight of the fungus garden and the height/circumference of the mounds was carried out using a statistical package 'Statistix 4.1'.

## RESULTS

Direct counting of mounds recorded a density of 8–10/ha. Mounds varying in height from 2–27 cm and 11.9–115 cm in circumference at the base, contained a population ranging from 3,900–70,077 individuals (Table 1). A mound with a height of 14 cm and 72 cm in circumference at the base has a population of  $27,042 \pm 20,664$  individuals, comprising  $60.68 \pm 8.51\%$  nymphs,  $31.45 \pm 7.83\%$  workers,  $3.98 \pm 2.36\%$  pre soldiers and  $3.89 \pm 2.94\%$  soldiers. Highly significant positive correlation ( $p < 0.01$ ) was obtained between the population and the height/circumference of the mound ( $Y = 4193.537 \times 1.111^X$  and  $Y = 3127.85 \times 1.026^X$ ). However two nests (nos. 6 and 8) recorded unexpectedly low populations of 23,400 and 28,911 individuals. A positive linear correlation ( $p < 0.01$ ) was obtained between the weight of the fun-

gus comb and the height/circumference of the mound ( $Y = -4.74 + 0.0036 X$  and  $Y = -8.199 + 0.142 X$ ).

The present study also revealed seasonal fluctuation in the population (Table 2). High population (34,280–43,758 individuals) was recorded during June–October. During this period the percentage of workers (8.75–12.18%) was significantly lower ( $p < 0.05$ ) while the percentage of larvae (67.9–81.6%) was high inside the nest. The proportion of soldiers and pre-soldiers did not show significant variation during the different months of the year. Mature alates were recorded during March, April, September and October whereas sexual nymphs were found throughout the year.

### DISCUSSION

Basalingappa (1976) estimated the population of *O. assmuthi* to vary from 23,672–530,765 individuals per nest while Rajagopal (1986) estimated the total population of *O. wallonensis* to vary from 74,793–423,552 individuals. Darlington (1990) also observed similar trends in population in *Macrotermes* mounds. In the present investigation population of *O. brunneus* varied from 3,900–70,077 individuals.

According to Darlington and Dransfield (1987), the population can be deduced fairly accurately from the nest parameters. So, it may be assumed that in *O. brunneus* a mound which is  $14.7 \pm 8.28$  cm high and  $72.19 \pm 35.02$  cm in circumference at the base will have a total sterile population of  $27042 \pm 20664$  individuals. The nymphs or larvae formed the bulk of the population, followed by workers and soldiers. The variation in the numbers of soldiers and presoldiers was not significant ( $p > 0.01$ ). Similar results have also been obtained by Collins (1981). However Agarwal (1976) recorded a higher percentage of workers (75%) than nymphs (25%) in *O. microdentatus*.

There seems to be a direct relationship between the size of the mound and number of termites in it (Howard *et al.*, 1982). In the present study total population increased corresponding to the size of the mound coupled with increase in the weight of the fungus comb. This is probably necessary for providing nutrition and accommodation to the growing population. Reason for the two nests recording unexpectedly low population is not known and they are considered to be aberrant.

When there is only one main wet season in the year, termites produce only one brood of alates (Collins, 1981) whereas where there are two well marked wet seasons, they produced either one or two broods (Lepage, 1984). In Kerala, there are three well-marked seasons every year—the pre-monsoon period (March–May), monsoon period (June–August) and post-monsoon period (October–November). *O. brunneus* swarms twice in a year during March/April and September/October (Miranda and Prabhoo, 1990). Mature alates were recorded in the nest during March, April, September and October whereas sexual nymphs were found throughout the year. Rajagopal (1986) observed alate nymphs of *O. wallonensis* to occur during September–March, whereas Thakur (1985) recorded sexual nymphs in *O. obesus* from January to August.

In *Macrotermes michaelsoni* the sterile populations are not affected in size or composition by the annual rearing of a reproductive brood, or by normal seasonal variations in climate and food availability (Darlington, 1986). She attributed this

TABLE 1. Population density of *Odonotermes brunneus*

| Height of mound (cm) | Cir. of mound (cm) | Wt. of fungus garden (g) | No. of insects in 100 g of fungus garden |            |              |                | Total (all castes) | Total no. of insects in the nest |
|----------------------|--------------------|--------------------------|--|------------|--------------|----------------|--------------------|----------------------------------|
|                      |                    |                          | Workers                                  | Soldiers   | Nymphs       | Soldier Nymphs |                    |                                  |
| 2                    | 11.9               | 250                      | 400 ± 4.80                               | 80 ± 3.41  | 950 ± 12.51  | 130 ± 2.61     | 1560               | 3900                             |
| 6                    | 33.62              | 300                      | 620 ± 5.61                               | 100 ± 2.62 | 1300 ± 15.61 | 80 ± 3.70      | 2100               | 6300                             |
| 8                    | 38.97              | 342                      | 920 ± 7.65                               | 120 ± 1.48 | 1850 ± 11.62 | 50 ± 1.82      | 2900               | 9918                             |
| 10                   | 61.6               | 436                      | 1500 ± 6.95                              | 200 ± 3.61 | 2150 ± 11.11 | 270 ± 1.61     | 4120               | 17963                            |
| 13                   | 72.62              | 515                      | 1400 ± 5.90                              | 500 ± 5.76 | 2450 ± 15.61 | 250 ± 2.71     | 4660               | 23999                            |
| 16                   | 82.0               | 650                      | 1310 ± 5.15                              | 180 ± 3.11 | 1960 ± 10.60 | 150 ± 1.61     | 3600               | 23400                            |
| 19                   | 96.1               | 680                      | 1950 ± 7.60                              | 70 ± 1.71  | 3100 ± 9.22  | 80 ± 2.71      | 5200               | 35360                            |
| 22                   | 100.6              | 749                      | 500 ± 5.25                               | 170 ± 3.61 | 3150 ± 13.45 | 140 ± 1.61     | 3860               | 28911                            |
| 24                   | 109.49             | 755                      | 2140 ± 8.71                              | 100 ± 2.11 | 4250 ± 14.23 | 210 ± 2.72     | 6700               | 50585                            |
| 27                   | 115.50             | 987                      | 2920 ± 6.72                              | 100 ± 1.92 | 4000 ± 12.61 | 80 ± 1.11      | 7100               | 70077                            |

Values are mean ± S D of 5 observations

TABLE 2. Seasonal population density in *Odontotermes brunneus*

| Months    | Wt of fungus garden (g) | Population density in 100 g of fungus garden |              |                   |              |                    |                   |                    |        |       |      | Total (in the nest) | Percentages |      |      |  |  |  |
|-----------|-------------------------|--|--------------|-------------------|--------------|--------------------|-------------------|--------------------|--------|-------|------|---------------------|-------------|------|------|--|--|--|
|           |                         | Workers (W)                                  | Soldiers (S) | Pre-soldiers (PS) | Larvae (L)   | Reproductive Brood |                   | Total (All castes) | W      | S     | PS   |                     | L           | SN   | A    |  |  |  |
|           |                         |  |              |                   |              | Sexual Nymphs (SN) | Mature alates (A) |                    |        |       |      |                     |             |      |      |  |  |  |
| January   | 780 ± 1.42              | 980 ± 4.81                                   | 120 ± 1.20   | 70 ± 1.11         | 2186 ± 14.81 | 100 ± 1.21         | -                 | 3456               | 26957  | 28.35 | 3.47 | 2.03                | 61.25       | 2.90 | -    |  |  |  |
| February  | 949 ± 1.51              | 850 ± 3.76                                   | 95 ± 1.50    | 80 ± 1.41         | 2140 ± 15.62 | 80 ± 1.15          | -                 | 3245               | 29946  | 26.19 | 2.93 | 2.47                | 65.95       | 2.47 | -    |  |  |  |
| March     | 955 ± 2.62              | 730 ± 4.15                                   | 110 ± 2.10   | 75 ± 1.62         | 1910 ± 13.88 | 150 ± 1.50         | 30 ± 2.81         | 3275               | 31276  | 22.29 | 3.36 | 2.29                | 58.32       | 4.58 | 9.16 |  |  |  |
| April     | 790 ± 2.16              | 960 ± 3.81                                   | 150 ± 1.76   | 83 ± 1.51         | 2210 ± 14.16 | 120 ± 1.62         | 250 ± 1.62        | 3773               | 29807  | 25.44 | 3.98 | 2.20                | 58.57       | 3.18 | 6.63 |  |  |  |
| May       | 860 ± 1.91              | 770 ± 4.61                                   | 125 ± 1.50   | 100 ± 2.11        | 1980 ± 15.86 | 80 ± 1.44          | -                 | 3055               | 26173  | 25.20 | 4.09 | 3.27                | 64.81       | 2.62 | -    |  |  |  |
| June      | 875 ± 2.65              | 480 ± 3.5                                    | 169 ± 2.62   | 95 ± 1.52         | 3510 ± 14.12 | 50 ± 1.21          | -                 | 4304               | 37660  | 11.15 | 2.49 | 1.40                | 81.6        | 0.74 | -    |  |  |  |
| July      | 959 ± 2.82              | 501 ± 4.61                                   | 200 ± 1.15   | 96 ± 1.16         | 3250 ± 13.65 | 65 ± 1.62          | -                 | 4112               | 39434  | 12.18 | 3.08 | 1.48                | 79.04       | 1.0  | -    |  |  |  |
| August    | 910 ± 1.88              | 372 ± 2.21                                   | 100 ± 1.90   | 120 ± 1.14        | 3050 ± 14.82 | 125 ± 1.45         | -                 | 3767               | 342780 | 9.88  | 1.58 | 1.9                 | 80.96       | 1.98 | -    |  |  |  |
| September | 975 ± 3.62              | 490 ± 2.21                                   | 220 ± 2.62   | 83 ± 1.90         | 3050 ± 15.15 | 75 ± 1.20          | 570 ± 1.55        | 4488               | 43758  | 10.9  | 3.18 | 1.2                 | 67.9        | 1.09 | 8.2  |  |  |  |
| October   | 855 ± 2.91              | 412 ± 2.61                                   | 211 ± 2.71   | 110 ± 2.4         | 3320 ± 14.62 | 68 ± 1.10          | 590 ± 2.76        | 4711               | 40279  | 8.75  | 2.72 | 1.42                | 70.47       | 0.88 | 7.6  |  |  |  |
| November  | 775 ± 3.45              | 1260 ± 2.16                                  | 168 ± 1.98   | 97 ± 1.77         | 1900 ± 12.71 | 100 ± 1.66         | -                 | 3525               | 30139  | 35.74 | 4.7  | 2.75                | 53.90       | 2.84 | -    |  |  |  |
| December  | 800 ± 2.91              | 1320 ± 5.92                                  | 159 ± 2.23   | 115 ± 0.41        | 2085 ± 12.56 | 116 ± 1.36         | -                 | 3789               | 30812  | 34.84 | 4.2  | 3.04                | 55.03       | 2.9  | -    |  |  |  |

Values are mean ± S.D of 6 observations

striking degree of stability to the sophisticated homeostatic mechanisms which maintain the nest temperature constant, the internal humidity at saturation and the gas composition of the air inside the nest constant. Sen-Sarma and Mishra (1969) while studying seasonal fluctuations in the nest population of *Microcerotermes beelsoni* explained that on account of the high density of alate nymphs during April, very active foraging was necessary for the maintenance of the improved nutrition for development of the young stages to maturity and a decline of worker and soldier population took place due to predation by ants and other natural enemies.

Agarwal (1976) reported a higher proportion of larvae during March–August and a higher proportion of workers from September–February in *O. microdentatus*. The soldier caste did not show much variation during the different months. He also reported high worker ratio and low larval numbers during July, October, November, December, January and February in *O. obesus*. Veeranna (1984) while studying seasonal fluctuation in the population of *O. obesus* and *O. wallonensis*, observed low population density during the months of November to May in *O. obesus*, whereas in *O. wallonensis* the population density was low during the months of December to July.

The present study showed high population during June–October in *O. brunneus*. During this period the proportion of workers was significantly lower while the percentage of larvae was high inside the nest. Low percentage of workers may be due to the fact that in *O. brunneus* foraging is increased during the rainy months resulting in reduced number of workers inside the nest. Higher percentage of larvae during June, July and August can be attributed to peak egg production in the monsoon season. However, the proportion of soldiers and pre-soldiers did not show significant variation during the different months of the year. The presence of the reproductive brood did not affect the sterile brood in this species. The weight of the fungus comb was closely related to the size of the sterile brood and did not vary seasonally as in *Odontotermes* sp. or *Microtermes* sp. which showed a reduction in comb weight during dry seasons (Wood *et al.*, 1977; Darlington, 1986).

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#### REFERENCES

- Agarwal, V. B. (1976) Seasonal fluctuations of proportions of different castes as found in the fungus combs of *Odontotermes obesus* and *O. microdentatus* (Roonwal and Sen Sarma). *Proceedings of Soil Biology and Ecology* **22**: 192–196.
- Akthar, M. S. and Rashid, I. M. (2001) Studies on population density and diversity of termites of District Bahawalnagar. *Pakistan Journal of Zoology* **12**(2): 95–104.
- Basalingappa, S. (1976) Sociotomy, a process of colony formation in the termites *Odontotermes assmuthi* Holmgren and *Speculitermes cyclops* (Roonwal and Sen Sarma) (Isoptera: Termitidae). *Journal of Karnataka University* **XXI**: 251–253.
- Collins, N. M. (1981) Populations, age structure and survivorship of colonies of *Macrotermes belli cosus* (Isoptera: Termitidae). *Journal of Animal Ecology* **50**: 293–311.

- Darlington, J. P. E. C. and Dransfield, R. D. (1987) Size relationships in nest populations and mound parameters in the termite *Macrotermes michaelseni* in Kenya. *Insectes Sociaux* **34**: 165–180.
- Darlington, J. P. E. C. (1986) Seasonality in mature mounds of the termite *Macrotermes michaelseni* in Kenya. *Insectes Sociaux* **33**: 168–189.
- Darlington, J. P. E. C. (1990) Populations in nests of the termite *Macrotermes subhyalinus* in Kenya. *Insectes Sociaux* **37**(2): 158–168.
- Howard, R. W., Jones, S. C., Mauldin, J. K. and Beal, R. H. (1982) Abundance, distribution and colony size estimates for *Reticulitermes* sp. (Isoptera: Rhinotermitidae) in Southern Mississippi. *Environmental Entomology* **11**(6): 1290–1293.
- Lepage, M. G. (1984) Distribution, density and evolution of *Macrotermes bellicosus* nests. (Isoptera: Macrotermitinae) in the North East of Ivory Coast. *Journal of Animal Ecology* **53**: 107–117.
- Miranda, M. T. P. and Prabhoo, N. R. (1990) Swarming behaviour and colony establishment in *Odontotermes brunneus* (Hagen) (Isoptera: Termitidae). *Social Insects—an Indian Perspective* 193–196.
- Rajagopal, D. (1986) Biological activities of the mound building termite, *Odontotermes wallonensis* (wasmann) (Isoptera: Termitidae) in Karnataka. *Journal of Soil Biology and Ecology* **6**(1): 42–52.
- Sen-Sarma, P. K. and Mishra, S. C. (1969) Seasonal variations of nest populations in *Microcerotermes besoni* (Snyder). *Proceedings of the National Institute of Science India* **35**(5): 361–367.
- Sen-Sarma, P. K. and Mishra, S. C. (1977) Seasonal fluctuations of colony compositions, nest population and foraging in *Nasutitermes dunensis* Chatterjee and Thakur (Isoptera: Termitidae). *Indian Journal of Entomology* 110–115.
- Thakur, M. L. (1985) Observations on the swarming in nature of termites (Insecta: Isoptera) at Coimbatore (Tamil Nadu: India). *Annals of Entomology* **3**(2): 25–32.
- Veeranna, (1984) Nests and nesting pattern of termites from North Karnatak Region. *Ph.D Thesis*, Karnatak University.
- Wood, T. G., Johnson, R. A. and Ohiagu, C. E. (1977) Population of termites (Isoptera) in natural and agricultural ecosystems in southern Guinea Savanna and Mokwa, Nigeria. *Insectes Sociaux* **27**: 110–118.

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## Effect of temperature on life history characteristics of *Dinarmus basalis* (Rond.) (Hymenoptera: Pteromalidae), a parasitoid of *Callosobruchus maculatus* (F.)

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**ABSTRACT:** *Dinarmus basalis* (Rond.) was reared at constant temperatures of 15, 20, 25, 30 and 35 °C on 18–20 days-old-larva of *Callosobruchus maculatus* (F.) developing in seeds black gram (*Phaseolus mungo*). Daily production of variable progeny, sex ratio, adult survivorship and development time were determined to calculate net reproductive rate ( $R_0$ ), generation time ( $T$ ), and intrinsic rate of increase ( $r_m$ ). Female longevity was greatest at 15 °C (41.58d) and shortest at 35 °C (20.25d). Development time and its variance decreased with the increase of temperature. Mean number of progeny produced per female during the life-span increased from 12.32 at 15 °C to 201.09 at 35 °C. Intrinsic rate of increase was lowest at 15 °C (0.021 females per female per day) and highest at 35 °C (0.390),  $R_0$  increased from 6.64 females per female at 15 °C to 149.48 at 35 °C, and  $T$  decreased from 90.15 to 12.6d. The relatively higher developmental attributes of the parasitoid is indicative of their high temperature tolerance. © 2005 Association for Advancement of Entomology

**KEYWORDS:** Life history characteristics, *Dinarmus basalis*, *Callosobruchus maculatus*

### INTRODUCTION

The tolerance of a parasitoid to the physical environments is a key factor to evaluate its efficacy and potential (Tillman and Powell, 1991). In the poikilothermic organisms the metabolic rates are proportional to the environmental temperatures (Andrewartha and Birch, 1954; Kitching, 1977; Wagner *et al.*, 1984).

*Callosobruchus maculatus* (F.) is one of the major pests of stored black gram and causes considerable losses. This pest is parasitized in their larval-pupal stages by the pteromalid parasitoid, *Dinarmus basalis* (Southgate, 1979). Biology of this parasitoid has been studied (Islam, 1995). But no data on the developmental characteristics

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as influenced by different temperature levels are available for *D. basalis*, on *C. maculatus*. The developmental parameters determined were female survivorship, age specific fecundity, sex ratio and development period.

### MATERIALS AND METHODS

Black gram, *Phaseolus mungo* L. used as a food medium were thoroughly washed, carefully dried under the sun and stored in air-tight containers. Twenty mated females of *C. maculatus* were released in individual Petri dishes (11.5 cm diam) containing fresh black gram seeds for oviposition. After one hour, the beetles were removed and the egg-containing seeds were placed in an incubator for rearing. When the developing larvae of *C. maculatus* were 20-22-days-old, they were used for the present investigation.

A freshly emerged and mated female parasitoid was introduced in a small petri dish (8.5 cm) containing 25 seeds infested by *C. maculatus* 20–22–days earlier. Fifteen different petri dishes with the constant number and constant host ages were maintained separately for a single mated female parasitoid for oviposition upto 24 h at 15 °C in an incubator. After 24 h, the female was removed. The seeds were changed and the females were again introduced for oviposition. The parasitized seeds were reared for development. The process was continued until death of the parasitoid. The same procedures were applied for 20, 25, 30 and 35 °C. Emerging progeny from parasitized seeds were recorded and removed daily. Thus, the number of progeny surviving to adulthood was used as a measure of age specific fecundity, which is valid for the calculation of life history statistics (Smith, 1992). Life history statistics were calculated followed by Birch (1948) and Deevey (1947). The data on temperature effect, age-specific fecundity, adult survivorship, fecundity and developmental time were analysed by the analysis of variance procedures.

The effects of temperature and mother's age on progeny sex ratio were analyzed by linear regression, using daily observations of percentage female for each mother, transformed by arsine square root.

### RESULTS

The length of *D. basalis* life varied inversely with temperatures. The female longevity was 41.58, 36.15, 26.32, 24.45 and 20.25 days at 15, 20, 25, 30 and 35 °C respectively. Survivorship of both adult males and females ( $l_x$ ) were highest at 15 °C and lowest at 35 °C (Fig. 1). Survivorship curves differed significantly at all temperature.

Age-specific production of female progeny ( $m_x$ ), as measured by number of female progeny surviving to adult emergence, is presented in Fig. 2. Large number of *D. basalis* were produced at 35 °C but small, at 15 °C. Peak daily fecundity increased with temperature (0.18, 0.72, 3.80, 8.35 and 10.98 females per day between days 10–40 at 15 °C, days 6–30 at 20 °C days, 2–20 at 25 °C, days 1–15 at 30 °C and days 1–10 at 35 °C, respectively) and the duration of the peak generally decreased with temperature.

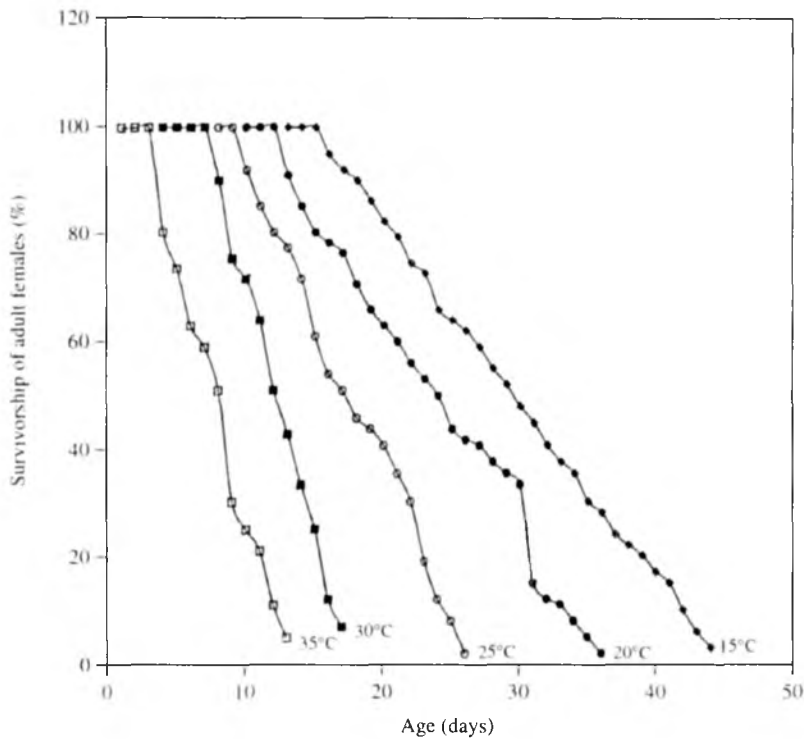


FIGURE 1. Survivorship of adult females of *D. basalis* exposed to *C. maculatus*.

Total fecundity over a female's life time, as measured by number of progeny surviving to adult emergence, increased with increasing temperature. Mean numbers of progeny per female at 15, 20, 25, 30 and 35 °C were 12.32, 62.35, 154.34, 180.58 and 201.09, respectively, and the results are presented in Table 1.

Progeny sex ratio was significantly lower at 15 °C and 20 °C (36.22 and 52.12% females) than at 25, 30 and 35 °C (67.38, 69.47 and 66.2% females, respectively) (Fig. 3, Table 1).

Mean developmental time and its variance increased with decreasing temperatures (Fig. 4). *D. basalis* completed development at all temperatures. In all cases males emerged earlier than females. The mean developmental times of females were  $42.4 \pm 2.90$ ,  $30.20 \pm 3.50$ ,  $22.10 \pm 1.70$ ,  $13.85 \pm 1.80$ ,  $12.05 \pm 1.50$  days and males were  $35.30 \pm 2.15$ ,  $26.10 \pm 2.40$ ,  $20.5 \pm 2.10$ ,  $12.08 \pm 1.32$ ,  $11.05 \pm 0.95$  days at 15 to 35 °C, respectively. The developmental time was longest at 15 °C and shortest at 35 °C, in both sexes.

Life history summary statistics are given in Table 1. Total progeny, net reproductive rate ( $R_0$ ), intrinsic rate of natural increase ( $r_m$ ), and finite rate of natural increase ( $\lambda$ ) were greatest at 35 °C. Generation time ( $T$ ), life expectancy of females at adult

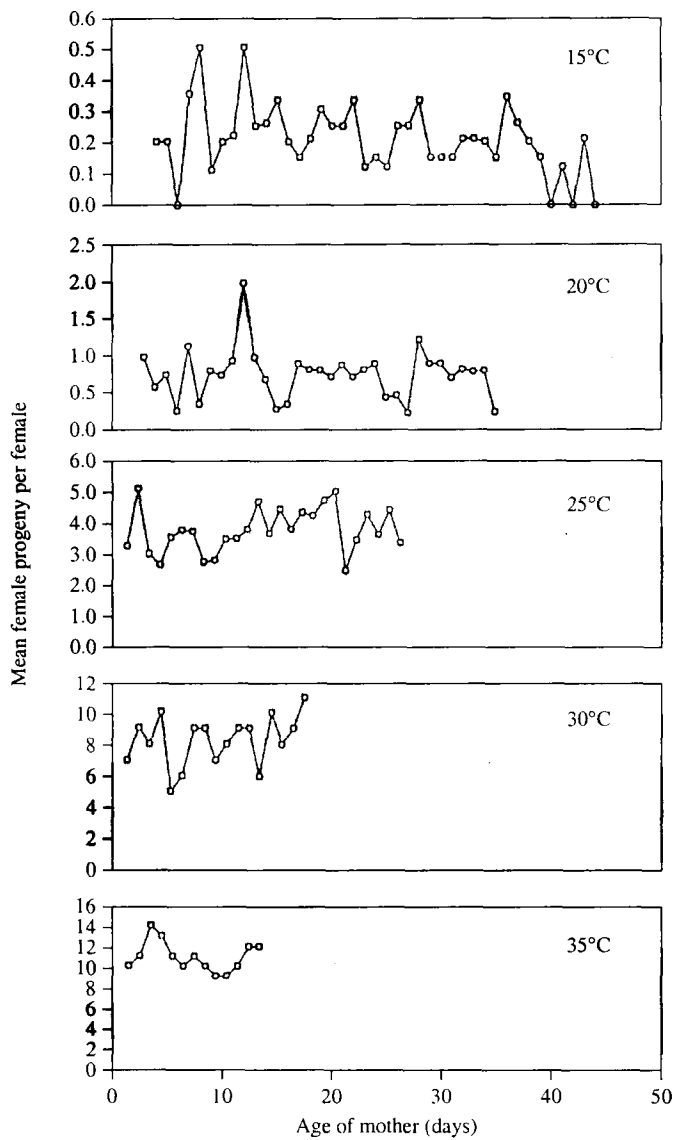


FIGURE 2. Age-specific production of female progeny by *D. basalis* exposed to *C. maculatus*. emergence ( $e_{adult}$ ), pre-oviposition period and population doubling time were greatest at 15 °C.

## DISCUSSION

Temperature and humidity are usually two of most important abiotic factors affecting the population dynamics of insects in storage system (Sinha, 1973; Flinn and

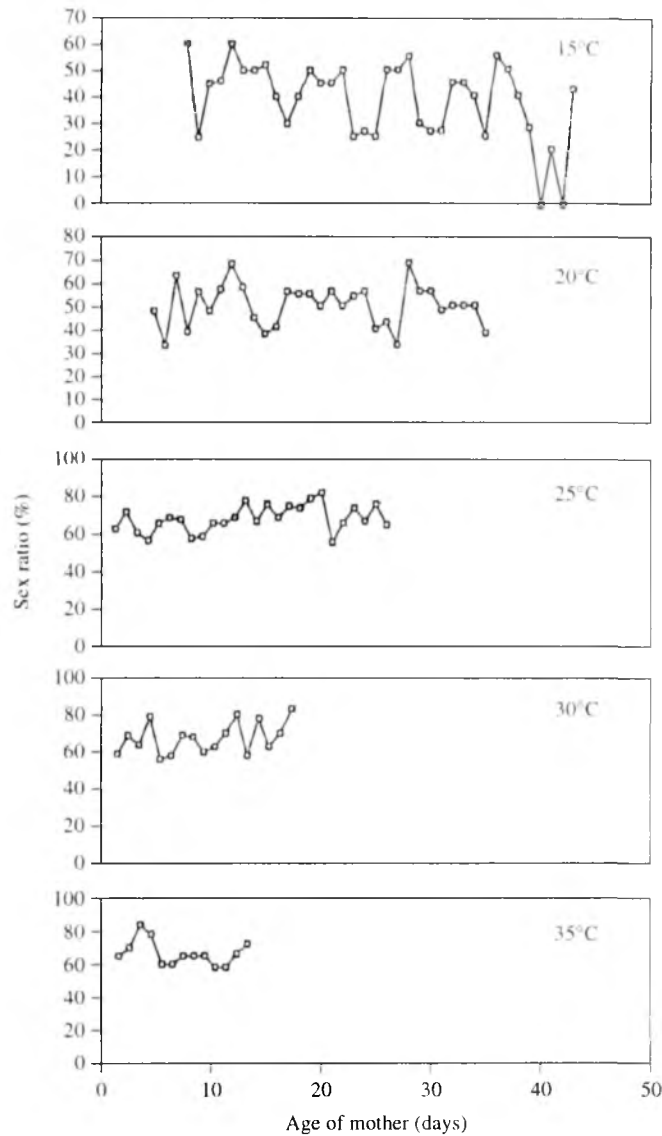


FIGURE 3. Sex ratio of *D. basalis* progeny in relation to age of mother (after arcsine-square root transformation).

Hagstrum, 1990). A limited number of studies have examined the changes in parasitoid life-history characteristics as a function of temperature and humidity (Messenger, 1968; Smith, 1992). Variations in temperature can affect searching behavior, parasitism rate (Mack *et al.*, 1981; Flinn, 1991), and the functional response (Butnett, 1954; Messenger, 1968; Mack *et al.*, 1981; Flinn, 1991).

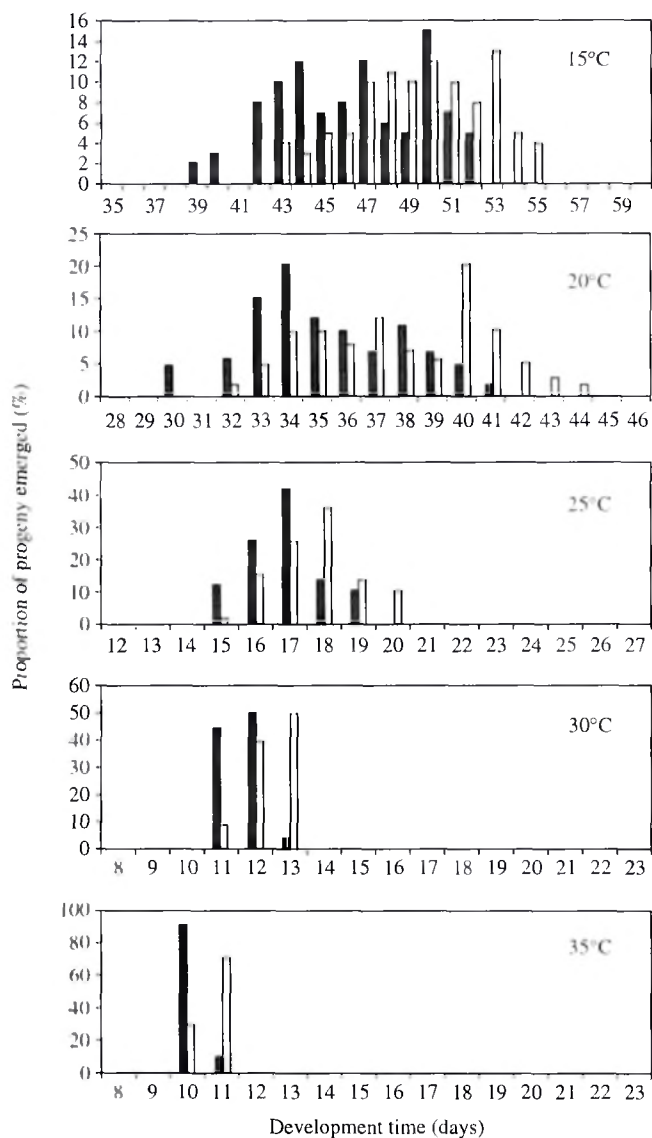


FIGURE 4. Distribution of development time of *D. basalis* from oviposition to adult emergence in relation temperature. ■ male, □ female.

In the present experiments the mean number of  $F_1$  progeny of *D. basalis* at 15–35 °C were 12.32, 62.35, 154.34, 180.58 and 201.09. The progeny production was extremely low at lower temperature and high at higher one. Okamoto (1972) noted that the emergence of *Anisopteromalus calandrae* was highest at 30 °C. Smith and Rutz

TABLE 1. Life history statistics of *D. basalis* in relation to temperature on *C. maculatus*

| Temperature<br>°C | Total<br>progeny | $R_0$  | Sex ratio<br>(% Female) | $T$    | $r_m$ | $\lambda$ | Pre-ovi-<br>position<br>period<br>(days) | Population<br>doubling<br>time<br>(days) |
|-------------------|------------------|--------|-------------------------|--------|-------|-----------|--|--|
| 15                | 12.32            | 4.46   | 36.22                   | 71.197 | 0.021 | 1.021     | 7.3                                      | 33.00                                    |
| 20                | 62.35            | 32.50  | 52.12                   | 46.416 | 0.075 | 1.077     | 3.5                                      | 9.24                                     |
| 25                | 154.34           | 104.00 | 67.38                   | 25.104 | 0.185 | 1.203     | 0.00                                     | 3.75                                     |
| 30                | 180.58           | 125.45 | 69.47                   | 13.884 | 0.348 | 1.416     | 0.00                                     | 1.99                                     |
| 35                | 201.09           | 133.95 | 66.12                   | 12.557 | 0.390 | 1.476     | 0.00                                     | 1.77                                     |

$R_0$  = Net reproductive rate;  $T$  = Generation time (days);  $r_m$  = Intrinsic rate of natural increase;  $\lambda$  = Finite rate of natural increase.

(1987) reared *Urolepis ruffipes* a pteromalid parasitoid on house flies at 15, 20, 25, 30 and 34 °C to measure daily fertility, fecundity and adult survivorship and found little production occurred at 15 °C and only a few female successfully emerged at 34 °C. The intrinsic rate of growth was faster at 30 °C (0.282) but fecundity was highest (165.5 hosts attacked) at 25 °C producing 124.5 progeny. Smith (1992) reported that the mean number of progeny produced per female of *A. calandrae* over its life time increased from 10.4 at 20 °C to 42.6 at 35 °C. The progeny sex ratio was much lower at 20 °C (33% female) than 25, 30 and 35 °C (55–68% female).

Variation in development time increased with decreasing temperature and larger than expected based on the general relationship for insects and mites as observed by Shaffer (1983). It also found that the development period of the females was longer than the males. The faster development of the male is widespread among parasitoids (Yeargan, 1983; Butler *et al.*, 1983; Hurlbult, 1987; Miura, 1989) and the phenomenon is called protandry (Wiklund and Fagerstorn, 1977).

Chun *et al.* (1992) observed the development time of *A. calandrae* at 6 constant temperatures in the host *Sitophilus oryzae* in laboratory condition, and reported that the development was inhibited at 18 °C, and at 18–34 °C the total development period decreased from 63.5 to 10.3 days in females and 72.0 to 9.9 days in males. Smith (1992) reported that in *A. calandrae* females, longevity was greatest at 20 °C and shortest at 30 and 35 °C. These reports indicate that the developmental period decreased with the increase of temperature, as found in this study.

The intrinsic rate of natural increase ( $r_m$ ), is the best single parameter for comparing potential population growth rates of different species (Andrewartha and Birch, 1954) but few data exist for hymenopteran parasitoids (Coats, 1976; Force, 1970). The values of  $r_m$  (0.021, 0.075, 0.185, 0.348, 0.390 at 15–35 °C respectively) are similar to those of *A. calandrae* females (0.028, 0.125, 0.194, 0.237, 0.250, 0.244 at 20–35 °C respectively) (Smith, 1992).

*D. basalis* is found to be an efficient parasitoid of *C. maculatus* infesting black gram seeds in stores and could be used for biological control in view of its tolerance to the relatively higher levels of ambient temperatures of 30 and 35 °C.

## ACKNOWLEDGEMENTS

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## REFERENCES

- Andrewartha, H. G. and Birch, L. C. (1954) *The Distribution and Abundance of Animals*. University of Chicago Press: Chicago, 782.
- Birch, L. C. (1948) The intrinsic rate of natural increase of an insect population. *Journal of Animal Ecology* **17**: 15–26.
- Butnett, T. (1954) Influence of natural temperatures and controlled host densities on oviposition of an insect parasite. *Physiological Zoology* **27**: 239–248.
- Butler, G. D. Jr, Hamilton, A. G. and Lopez, J. D. Jr (1983) *Cardiochiles nigriceps* (Hymenoptera: Braconidae): Development time and fecundity in relation to temperature. *Annals of Entomological Society of America* **76**: 536–538.
- Coats, S. A. (1976) Life cycle and behaviour of *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). *Annals of Entomological Society of America* **69**: 772–780.
- Chun, Y. S., Yoon, T. J., Shin, S. S. and Ryoo, M. I. (1992) Relationship between temperature and development of an ectoparasitoid of rice weevil (Curculionidae: Coleoptera), *Anisopteromalus calandrae* (Pteromalidae: Hymenoptera). *Korean Journal of Entomology* **227**: 297–303.
- Deevey, E. S. Jr. (1947) Life tables for natural populations of animals. *Quarterly Review of Biology* **22**: 283–314.
- Flinn, P. W. (1991) Temperature dependent functional response of the *Cephalonomia waterstoni* (Hym. Bethyilidae) attacking rusty grain beetle larvae. *Environmental Entomology* **20**: 872–876.
- Flinn, P. W. and Hagstrum, D. W. (1990) Simulations comparing the effectiveness of various stored grain management practices used to control *Rhyzopertha dominica*. *Environmental Entomology* **19**: 725–729.
- Force, D. C. (1970) Competition among four hymenopterous parasites of an endemic insects host. *Annals of Entomological Society of America* **63**: 1975–1988.
- Hurlbult, B. L. (1987) Sexual size dimorphism in parasitoid wasps. *Biol. J. Linn. Soc.* **30**: 63–89.
- Islam, W. (1995) Biology of *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae), a larval-pupal parasitoid of *Callosobruchus chinensis* (Linnaeus). *Annals of Entomology* **13**: 51–56.
- Kitching, R. G. (1977) The resources and population dynamics of insects. *Australian Journal of Ecology* **2**: 31–42.
- Mack, T. P., Bajusz, B. A., Nolan, E. S. and Smilowitz, S. (1981) Development of a temperature-mediated functional response equation. *Environmental Entomology* **10**: 573–579.
- Messenger, P. S. (1968) Bioclimatic studies of the aphid parasite *Praon exsoletum*. Effects of temperature on the functional response of females to varying host densities. *Canadian Entomology* **100**: 728–740.
- Miura, K. (1989) Effect of temperature on the development of *Gonatocerus cinticipitis* Sahad, an egg parasitoid of green rice leafhopper. *Applied Entomology Zoology* **25**: 146–147.
- Okamoto, K. (1972) The synchronization of the life cycle between *Callosobruchus chinensis* (L.) and its parasite *Anisopteromalus calandrae* (Howard) II. The relationship between the development of the parasite and the developmental stage of the host. *Japanese Journal of Ecology* **22**: 238–244.

- Sinha, R. N. (1973) Interrelations of physical, chemical and biological variables in the deterioration of stored grains. In: *Grain Storage Part of System*, Sinha, R. N. and Muvi, W. E. (Eds). Westport Avi
- Smith, L. (1992) Effect of temperature on life history characteristics of *Anisopteromalus calandrae* parasitizing maize weevil larvae in corn kernels. *Environmental Entomology* **21**: 877–887.
- Smith, L. and Rutz, D. A. (1987) Reproduction, adult survival and Intrinsic rate of growth of *Urolepis rufipes* (Hymenoptera: Pteromalidae), a pupal parasitoid of house flies, *Musca domestica*. *Entomophaga* **32**: 315–327.
- Southgate, B. J. (1979) Biology of the Bruchidae. *Annual Review of Entomology* **24**: 449–473.
- Shaffer, P. L. (1983) Prediction of variation in development period of insects and mites reared at constant temperatures. *Environmental Entomology* **12**: 1012–1019.
- Tillman, P. G. and Powell, J. E. (1991) Development time in relation to temperature for *Microplites croceipes*, *M. demolitor*, *Cotesia kazak* (Hymenoptera: Braconidae), *Hyposoter didymator* (Hymenoptera: Ichneumonidae), endoparasitoids of the tobacco budworm (Lepidoptera: Noctuidae). *Environmental Entomology* **20**: 61–64.
- Wagner, T. L., Wu, H., Sharpe, P. J. H., Schoolfield, R. M. and Coulson, R. N. (1984) Modeling insect development rates: a literature review and application of a biophysical model. *Annals of Entomology Society of America* **77**: 208–225.
- Wiklund, C. and Fagerstorn, T. (1977) Why do males emerge before female?. *Oecologia* **31**: 153–158.
- Yeargan, K. V. (1983) Effect of temperature on developmental rate of *Trissolcus euschisti* (Hymenoptera: Scelionidae), a parasite of sink bug eggs. *Annals of Entomological Society of America* **76**: 757–760.

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## Description of three new and record of two known species of Encyrtidae (Hymenoptera: Chalcidoidea) from Northeast India

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**ABSTRACT:** Three new species, *Metaphycus cassiae*, *Astymachus felix* and *Cheiloneurella indica* are described from Assam and Manipur. Genus *Cheiloneurella* is recorded from India for the first time. *Clausenia purpurea* Ishii and *Ooencyrtus corbetti* Ferriere are reported for the first time from India. *O. corbetti* is recorded from a new host, *Podontia affinis*. © 2005 Association for Advancement of Entomology

**KEYWORDS:** new species, new host record, *Metaphycus cassiae*, *Astymachus felix*, *Cheiloneurella indica*, *Clausenia purpurea*

### INTRODUCTION

This paper is a continuation of exploring the encyrtid fauna of the Northeast India undertaken by the authors since 1991. Present study is based on the collection made from Assam and Manipur by the first author. Here we describe three new species belonging to the genera *Metaphycus*, *Astymachus* and *Cheiloneurella*. Genus *Cheiloneurella* and two other species—*Clausenia purpurea* Ishii and *Ooencyrtus corbetti* Ferriere are reported from India for the first time. Types and other identified material are deposited with National Forest Insect Collection at Forest Research Institute, Dehra Dun (NFICFRI) and some material at Zoology Department, Aligarh Muslim University, Aligarh (ZDAMU). All the measurements used in descriptions are in  $\mu\text{m}$  unless mentioned otherwise.

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### Genus *Metaphycus* Mercet

This is large cosmopolitan genus with about 200 described species. In India the genus is reviewed by Zeya and Hayat (1993) and represented by thirteen species. They are parasitoids of several families of Coccidae, Eriococcidae and Asterolecaniidae (Homoptera), which are pests of many trees and shrubs of forestry, horticultural and ornamental importance. A new species, *M. cassiae*, is described which belongs to the *alberti*-group, characterized by two-segmented maxillary and labial palpi.

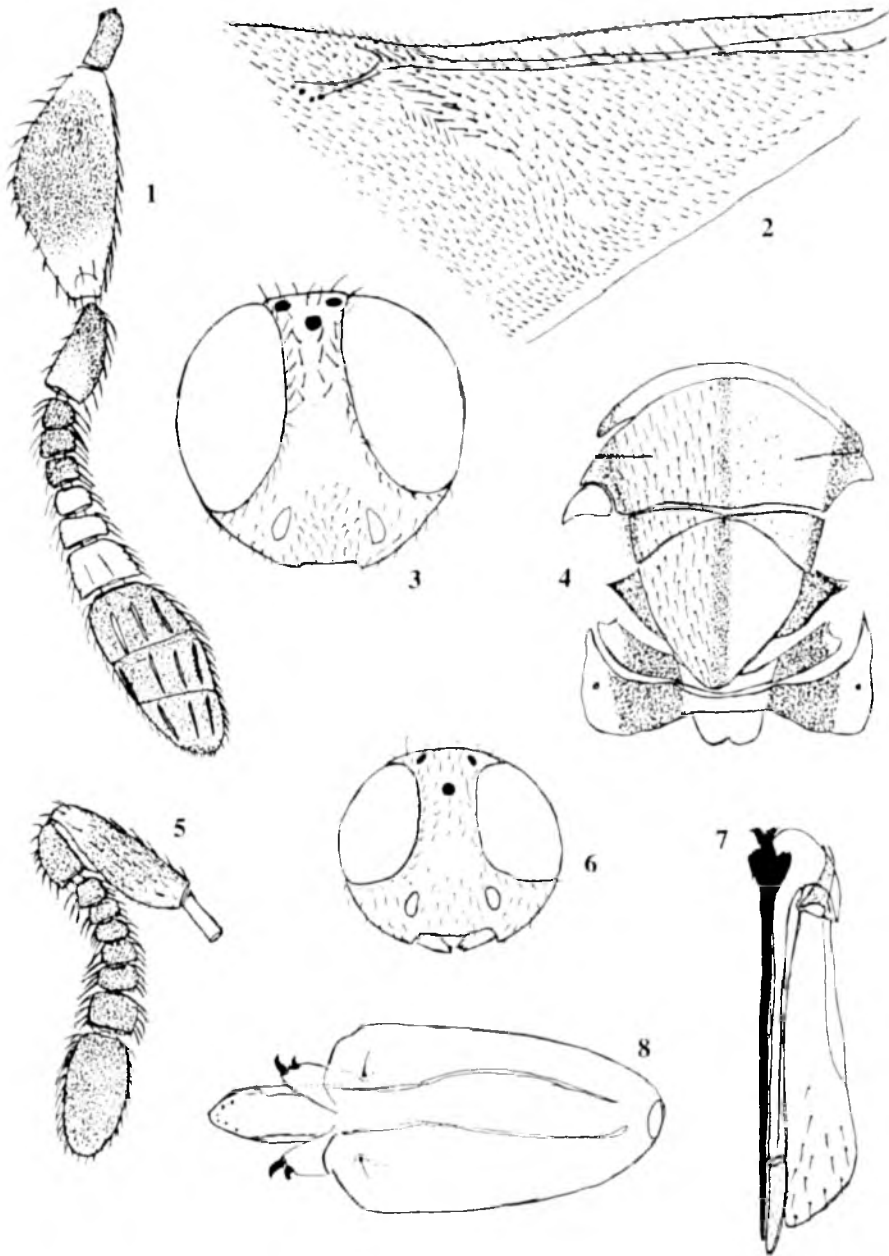
#### *Metaphycus cassiae* sp. nov. (Figs 1–8)

**Female.** Body length, without exerted part of ovipositor,  $0.75 \pm 0.037$  mm ( $n = 12$ , range 0.693 to 0.803 mm). Ovipositor exerted by 0.025 mm. Light yellow dorsally with three brown bands on thorax, two lateral and one median; ventrally body lighter. Setae all over the body white. Head, frontovertex yellow, ocelli red, eyes gray, lower portion of head lighter; malar area light yellow with dark brown patch on posterior side of malar sulcus; post occiput with a black spot in the middle. Antenna as in Fig. 1. Thorax (Fig. 4) yellow; pronotum light yellow; mesonotum and scutellum, medially with a longitudinal light brown band; sides of pronotum, mesoscutum, axillae, metanotum and propodeum on either side with light brown band all in a continuity and form the side bands of the thorax; bands on the metanotum and propodeum wider and more darker and restricted to inner side of propodeal spiracles. Wings hyaline. Legs light yellow. Gaster light brownish yellow, basal tergite with two dark spots one on each side and in line with those on the lateral bands of metanotum and propodeum.

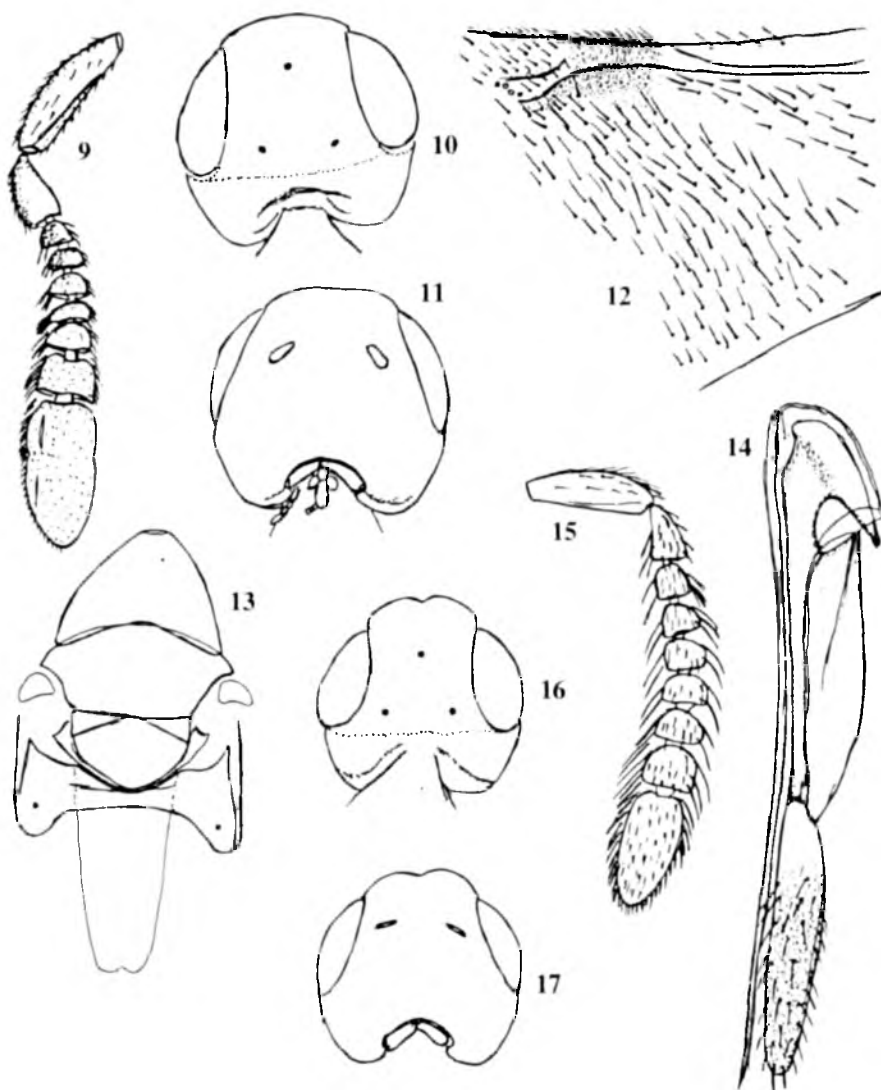
Head, in frontal view (Fig. 3) rounded, as long as wide, 198; frontovertex (51) slightly more than one-fourth the head width. Ocellar triangle obtuse, middle ocellus diameter, 22; posterior ocellar line, 22; ocello-collar line, 12.8; ocello-ocular line, 6.4. Toruli below the line joining lower margins of eye; torulus twice as long as wide, 25.6 : 12.8; inter toral distance, 51.2; torulo-mouth distance, 12.8; torulo eye distance 25.6. Scrobes deep their dorsal margin meeting; reaching half way between distance from torulus to middle ocellus, 70.4:134.4. Malar space 0.29x as long as head length, 57:198. Mandibles tridentate; labial and maxillary palpi 2-segmented each. Antenna with scape slightly longer than 2x its width, 96:44.8; pedicel 2.16x as long as wide (41.6:19.2); rest as in the Fig. 1.

Thorax (Fig. 4), flat dorsally, 1.25x as long as wide (378:301). Pronotum 16x as wide as long dorsally. Mesoscutum with incomplete parapsidal furrows; 1.7x as wide as long, 217:121; scutellum, as long as wide, as long as mesoscutum. Fore wing 2.55x as long as wide, 505:198. Marginal vein 25.6; post marginal, 12.8; stigmal, 38.4; Fig. 2. Hind wing 5.4x as long as wide, 345.6:64; vein length 230. Middle leg tibial spur as long as basitarsus, 70.4.

Gaster, wider than long, 301:275; shorter than thorax. Ovipositor slightly exerted, 17 shorter than mid tibia length, 179:192; II-valvifer, III-valvula and outer plate 137.6, 41.6 and 134.4 long, respectively; rest as in Fig. 7.

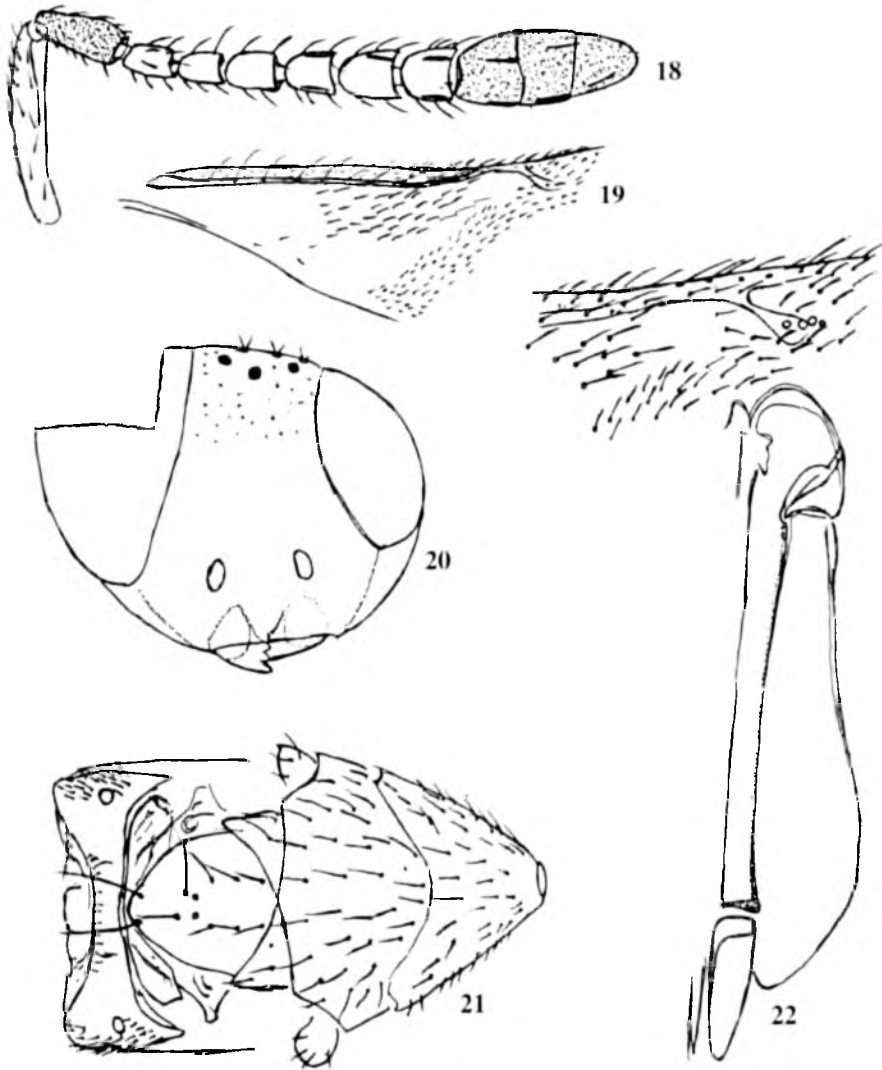


FIGURES 1–8: *Metaphycus cassiae* sp. nov., female: 1, antenna, 2, fore wing setation and venation at basal half; 3, head in frontal view; 4, thorax in dorsal view; 7, ovipositor. Male: 5, antenna; 6, head in frontal view; 8, genitalia.



FIGURES 9–17: *Astymachus felix* sp. nov., female: 9, antenna; 10, head in dorsal view; 11 head in frontal view; 12, fore wing setation and venation at basal half; 13, thorax in dorsal view; 14, ovipositor. Male: 15, antenna; 16, head in dorsal view; 17, head in frontal view.

**Male.** Body length  $0.56 \pm 0.043$  mm ( $n = 6$ , range 0.48 to 0.59 mm). Similar to female except that the brown areas on thorax wider and quite diffused into each other, thorax appear light brown with median line darker. Antenna with scape and pedicel lighter than funicle; scape tips lighter.



FIGURES 18–22: *Cheiloneurella indica* sp. nov., female: 18, antenna; 19a, fore wing showing setal pattern at basal half; 19b, fore wing venation enlarged; 20, head in frontal view; 21, thorax in dorsal view; 22, ovipositor.

Head, in frontal view (Fig. 6) rounded, wider than long, 153:140; frontovertex (51) one-third the head width. Ocellar triangle obtuse, middle ocellus diameter, 14.5; posterior ocellar line, 22.4; ocello-collar line and ocello-ocular line, each 4.8; Toruli below the line joining lower margins of eye; inter toral distance, 44.8; torulo-mouth

distance, 6.4; torulo eye distance 9.6. Scrobes deep their dorsal margin meeting; reaching half way between distance from torulus to middle ocellus, 70.4:134.4. Malar space 0.31x as long as head length, 44.8:140. Antenna with scape 3x as long as wide, 67:22; pedicel 1.9x as long as wide (30:16); rest as in the Fig. 5.

Thorax flat dorsally, 1.25x as long as wide (378:301). Mesoscutum 2x as wide as long, 176:86; scutellum wider than long, 102.4:92.8; longer than mesoscutum. Fore wing 2.34x as long as wide, 368:157. Marginal vein 14.5; post marginal, 4.8; stigmal, 27.2. Hind wing 5.4x as long as wide, 173:32; vein length 115. Middle leg tibia length, 144; mid-tibial spur slightly longer than mid-basitarsus, 51:48.

Gaster, with genitalia as in Fig. 8; aedeagus length, 64; phallobase length 61.

*Host.* Coccids on *Cassia tora*

*Distribution.* India: Assam.

Holotype. ♀, on card; India: Assam, Jorhat, Club Road; 20. viii. 1993; ex. Coccids on *Cassia tora*; Sudhir Singh. (NFICFRI NO. 21063).

Paratypes. 11♀, 6♂, on four cards, (1♀ and 1♂ dissected and mounted on a slide each), data same as for holotype (NFICFRI); 3♀ and 1♂ on two slides, data same as for holotype (ZDAMU).

*Comments.* This species is apparently closely related to *M. malabarensis* (Mukerjee, in Mukerjee *et al.* (1975). Additional characters based on the study of the holotype were given by Zeya and Hayat (1993). The new species differs from *malabaraensis* mainly in the following characters: Scape expanded, 2.5x as long as broad, with base narrowly and apical fifth pale; F1-3 brown; frontoververtex slightly broader than 0.25x of head width; ovipositor with III-valvulae about 0.3x of II-valvifer and longer than 0.5x of mid tibial spur. (In *malabarensis*: Scape, as figured by Mukerjee, 3x as long as broad, with basal 0.25 and apical 0.25 pale; F1-4 brown; frontoververtex at least about 0.25x of head width; ovipositor with III-valvula about 0.2x of second valvifer (13:70) and shorter than 0.5x of mid tibial spur (13:28).

*Etymology.* The species name is derived from plant genus name *Cassia* from which its coccid host was collected.

### Genus *Astymachus* Howard

Genus has distribution in Palaearctic and Oriental regions of the world with only three known species. From India only one species—*A. japonicus* Howard is recorded. Below we describe a new species based on material collected from Assam and Manipur.

#### *Astymachus felix* sp. nov. (Figs. 9–17)

[*Astymachus japonicus* Howard: Fatima & Shafee, 1994: 117, F. Fig. 22-E. India, Aligarh. Misidentification.]

**Female.** Body length, excluding exerted part of ovipositor,  $1.32 \pm 0.073$  (range 1.25–1.45) mm (Holotype, 1.32 mm). Exserted part of ovipositor  $0.16 \pm 0.013$ . Body yellow, more or less completely yellowish, indistinctly suffused pale brown on frons anterior to median ocellus; malar region and sides of eyes darker; third valvula dark brown. Antenna brown, dorsal surface of pedicel darker. Forewing pale, suffused brown, with a distinct dark patch around marginal vein, and a dark long streak in Basal half near posterior margin.

Head, round, hypognathous; slightly wider than long, 287:257. In dorsal view (Fig. 10) 2x as wide as frontovertex at middle ocellus level (141); ocelli in acute angle triangle. MOD, 13; posterior ocellar line, 77; ocello-collar line, 38; ocello-ocular line, 24. In frontal view (Fig. 11), inter torular distance, 77; torulo-eye distance, 38; torulo-mouth distance, 128. Head 2.2x as long as malar space, 115. Antenna with scape 3.5x as long as wide, 154:44; pedicel twice as long as wide, 80:40; rest as in Fig. 9.

Thorax (Fig. 13), with pronotum triangular 1.4x as wide as long (218:154); mesoscutum 2.5x as wide as long (257:103); scutellum 1.85x as wide as long (167:90); propodeum about a seventh of scutellum length; thoracic phragma 2.6x as long as scutellum (231:90). Basitarsus length 1.62x as long as spur. Fore wing (Fig. 12) 3.3x as long as wide (723:218); marginal vein slightly shorter than stigmal (51:64). Hind wing more than 7x as long as wide; 1.52x as long as vein length; marginal fringe of fore wing longer than that of hind wing.

Gaster. 2.2x as long as thorax (775:350), exerted part of ovipositor 0.22x gaster length (175:775). Ovipositor 0.66x as long as gaster 514:775; II-valvifer length 311; III-valvula length 193, rest as in Fig. 14.

**Male.** Body length,  $1.04 \pm 0.1$  mm (range 0.87–1.17 mm), similar to female in colouration. Head in dorsal view (Fig. 16) 1.7x as wide as frontovertex at middle ocellus level 244:128; posterior ocellar line, 51; ocello-collar line, 19; ocello-ocular line, 26. In frontal view (Fig. 17) inter torular distance, 77; torulo-eye distance, 22; torulo-mouth distance, 103. Antennae as in Fig. 15.

Thorax. Mesoscutum 2x as wide as long (231:116); scutellum 1.66x as wide as long (128:77); propodeum about a fifth of scutellum length. thoracic phragma 2.8x as long as scutellum (218:77).

Gaster. 1.5x as long as thorax (539:360), 1.75x as long as wide, 539:308.

*Host.* Unknown.

*Distribution.* India. Assam; Manipur; Uttar Pradesh.

Holotype: ♀, whole undissected mounted on a slide under a small cover slip with other paratypes: India: Assam, Haflong; 10.x.1987; (Sudhir Singh, coll.No.32) (NFICFRI No. 21064).

Paratypes: 6♀, 8♂, India: Assam, Haflong; 10.x.1987; (Sudhir Singh, coll. No.32). The slide has also under another small cover slip one male paratype, and under a large cover slip, 4♀, and 6♂ paratypes. The holotype is distinctively marked. Also one ♀, one

♂ (clava missing) and one dissected ♀ under several cover slip pieces (all paratypes) on a second slide (NFICFRI No. 21064).

Additional material, not designated as types: Manipur, Imphal, 1♀ (on slide); 27.x.1987; (Sudhir Singh, No. 70). Uttar Pradesh, Aligarh, 6♀ (on slide with parts of 1♂ on a second slide), 23.i.1985 (Fatima, ref. No. 944). Det. *Astymachus japonicus* Howard, by Fatima and Shafee (ZDAMU).

*Comments.* Hayat (1999) already noted that the specimen det. *A. japonicus* by Fatima and Shafee (1994) are misidentified. The specimens belonging to the same species were also collected from Assam and Manipur (coll. Sudhir Singh), and types are designated from the Assamese specimens.

*A. felix* sp. nov. agree with *A. phragmitis* Trjapitzin (1962) and *A. exilis* Prinsloo (1989) in having only F6 larger and F1-5 subequal in length and is broader than long. From both these species, *felix* differs in having clava with an incomplete suture; less than 20 (16–18) seta on the scutellum; 3-segmented labial palp; fore wing evenly pale infusate with a dark patch at marginal and stigmal veins, and a distinct infusate longitudinal strip in basal half parallel to posterior wing margin; the fore wing disc proximad of the linea clava rather densely setose; and the marginal fringe long, nearly 0.25x of wing width.

*Etymology.* The species name is derived from latin word *felix* meaning lucky, fortunate, happy.

### Genus *Cheiloneurella* Girault

Genus is described from Australia with only one known species. Noyes and Hayat (1984) have mentioned of its distribution in India, Thailand, Hong Kong, Malaysia, Indonesia and Philippines. It is reported from India with description of a new species *C. indica* collected from Assam.

#### *Cheiloneurella indica*, sp. nov. (Figs 18–22)

Female. Length, about 1.2 mm. Body largely golden yellow; mouth margin dark brown; pronotum anteriorly and side of axillae brownish; tegulae pale yellow; gaster with two pale brownish patches on T1, and a large, irregular dark reddish brown patch at each cercal plate. Mandible dark reddish brown at apical half. Scape pale yellowish brown; funicle pale yellow; pedicel and clava brownish. Wings hyaline; fore wing with a light yellow infuscation especially behind distal half of submarginal vein to apex of venation, the disc beyond venation hyaline; marginal vein dark brown. Legs, including coxa, pale yellow. Setae on frontovertex pale brown; eyes with short, transparent setae, each shorter than a facet; setae on pronotum, mesoscutum, axillae and scutellum brown; on sides of propodeum setae dense and whitish; gasteral terga with setae as follows: TI–IV each with a broadly interrupted line (3–4 setae on each side); TV with a complete line; TVI with a curved line of setae; TVII with several longer setae. Frontovertex with fine, irregular, polygonal reticulations; on face, scrobes and

malar space slightly longitudinally elongated reticulations; mesoscutum with irregular polytonally reticulate sculpture, the cells large and fine, visible at higher magnification; scutellum about same sculpture as on mesoscutum, but the cells relatively smaller; axillae with transversely elongate cells; T1 of gaster of each side in posterior half with distinct raised reticulations.

*Head.* (Fig. 20). round, 1.2x as wide as long, 373:308; 2.7x as wide as frontovertex (135); mouth fossa about as broad as frontovertex width, 140:135. Toruli on line joining lower margins of eyes; torulus 1.8x as long as wide; torulo-mouth distance equal to torulo eye distance, 55; inter torular distance, 65; torulo-middle ocellus distance 3.27x torulo-mouth distance, 180:55. Scrobes meeting dorsally, shallow reaching about half way the torulo-mid ocellus distance. Ocelli in obtuse triangle; middle ocellus diameter, 15; posterior ocellus line, 60; ocello-ocular line, 25. Antenna with scape 6x as long as wide, 150:25; pedicel 2.4x as long as wide, rest as in Fig. 18.

Thorax (Fig. 21). 1.6x as long as wide, 514: 321. Pronotum triangular, 2.5x as wide as median length, 257: 103; mesoscutum 1.7x as wide as long, 308:180; scutellum slightly longer than wide, 167:154; propodeum about one-sixth of scutellum length, 26. Fore wing 3.25x as long as wide; marginal, post marginal and stigmal vein 65, 32 and 45 long, respectively (Fig. 19). Hind wing 6.2x as long as wide, 642:103; vein length 0.66x wing length, 424:642. Middle leg basitarsus slightly longer than middle tibial spur, 575:550.

Gaster. 1.2x as long as wide, 470:385, shorter than thorax. Hypopygium reaching 0.7x gaster length, 330:470. Ovipositor slightly exerted, 40. Ovipositor length, 411, II-valvifer 3.5x as long as III-valvula, 321:90; outer plate length, 308; rest as in Fig. 22.

*Male.* Unknown.

*Host.* Unknown.

*Distribution.* Indian: Assam.

Holotype: ♀, (on slide under three cover slips): India: Assam: Jorhat: Lohpohia; 2.ix.1994; (Sudhir Singh, coll. No. E32) (NFICFRI No. 21065).

*Comments.* This species is very close to the type species, *binotativentris* Girault (1915); see also Dhams and Gordh, 1997 for a redescription based on the remains of the type), but differs mainly in the following characters: Pronotum about 0.66x of mesoscutum length; propodeum about one-fifth of scutellum length; gaster sub-equal in length of thorax; and fore wing slightly more than 3x as long as broad with the stigmal vein emitted at a more acute angle with the wing margin. (In *binotativentris*: Pronotum 1.29x as long as mesoscutum; propodeum 0.25x of scutellum length; gaster about 0.8x of thorax length; and fore wing 2.86x as long as broad, with the stigmal vein emitted at relatively more obtuse angle with the wing margin.)

*Etymology.* The species name is derived from name of country of origin—India.

***Ooencyrtus corbetti* Ferriere**

Specimens examined: India: Assam, Jorhat, 20♀, 4♂, (one ♀ on slide), 10.viii.1994 (Sudhir Singh, No. E.6), ex. Eggs of *Podontia affinis* (Col.: Chrysomelidae). Det. By Sudhir Singh (2♀ in ZDAMU, rest in NFICFRI No. 21066).

This species is known so far only from Malaysia from eggs of *Podontia quatuordecimpunctata* (Coleoptera: Chrysomelidae) (Huang and Noyes, 1994). It is here recorded from India for the first time with a new host record.

***Clausenia purpurea* Ishii**

Specimen examined: India: Assam, Haflong, one ♀, (on card, with one antenna and forewing on a slide), 17–19.iv.1988 (Sudhir Singh, No. 88) Det. Sudhir Singh (In ZDAMU).

This species is being recorded from India for the first time.

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## REFERENCES

- Dhams, E. and Gordh, G. (1997) A review of the genera of Australian Encyrtidae (Hymenoptera: Chalcidoidea) described from Australia by A.A. Girault with a checklist of included species. In: *Memoirs on Entomology, International* Vol. 9, Associated Publishers: Vol 9.
- Fatima, A. and Shafee, S. A. (1994) Studies on the taxonomy of the Indian encyrtids (Hymenoptera: Encyrtinae). *Aligarh Muslim University Publication (Zoological Series) on Indian Insect Types* 15: 116–117.
- Girault, A. A. (1915) Australian hymenoptera Chalcidoidea-VII. The family Encyrtidae with description & of new genera and species. *Memoirs of the Queensland Museum* 4: 1–184.
- Hayat, M. (1999) Taxonomic notes on Indian Encyrtidae (Hymenoptera: Chalcidoidea)-V. *Oriental Insects* 33: 349–407.
- Huang, D. W. and Noyes, J. S. (1994) A revision of Indo-Pacific species of *Ooencyrtus* important insect species (mainly Hemiptera and Lepidoptera). *Bulletin of natural History Museum London (Entomology)* 63(1): 1–136.
- Mukerjee, M. K., Saraswat, G. G. and Mukerjee, M. K. (1975) Records of some known and descriptions of new species of chalcids (Hymenoptera) from India. *Memoirs of the School of Entomology, St. John's College* 4: 35–62.
- Noyes, J. S. and Hayat, M. (1984) A review of the genera of Indo-Pacific Encyrtidae (Encyrtidae: Chalcidoidea). *Bulletin of British Museum Natural History (Entomology)* 48: 131–395.
- Prinsloo, G. L. (1989) The southern African species of *Astymachus* Howard and *Rhopus* Forester (Hymenoptera: Encyrtidae). *J. Ent. Soc. South Africa* 52: 129–147.
- Trjapitzin, V. A. (1962) Encyrtidae (Hymenoptera) parasites of *Nipponocalerda turanica* (Arch.) (Homoptera, Acleridae) in Nogai steppe (In Russian). *Zool. Zhurnl* 41: 560–570.
- Zeya, S. B. and Hayat, M. (1962) A review of the Indian species of *Metaphycus* (Hymenoptera: Encyrtidae). *Oriental Insects* 27: 185–210.

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## ***Cohicaleyrodes indicus* (David and Selvakumaran) comb. n. and *Cohicaleyrodes jesudasani* nomen novum**

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**ABSTRACT:** An examination of the holotypes of *Mixaleyrodes indicus* David and Selvakumaran and *Cohicalerodes indicus* Jesudasan and David revealed that the former is to be assigned to the genus *Cohicaleyrodes* Bink suggesting a new combination. In view of the above a new name *Cohicaleyrodes jesudasani* has been proposed for *C. indicus* Jesudasan and David. © 2005 Association for Advancement of Entomology

**KEYWORDS:** *Cohicaleyrodes indicus* comb. n., *Cohicaleyrodes jesudasani*

### ***Cohicaleyrodes indicus* (David and Selvakumaran) comb. n.**

*Mixaleyrodes indicus* David and Selvakumaran, 1987: *J. Bombay Nat. Hist. Soc.*, **84**: 654–656. Host plant: *Litsea travancorica*, Idukki (Kerala), 13.06.1986, coll. S. Selvakumaran.

In 1987 David and Selvakumaran described a new species, *Mixaleyrodes indicus* collected by S. Selvakumaran at Idukki (Kerala State) on 13.06.1986 from *Listea travancorica*. While placing the species under the genus *Mixaleyrodes* they remarked “This species does not really fit into the generic characters of *Mixaleyrodes* due to absence of thoracic tracheal pores or clefts and folds.” A careful study of the holotype revealed that this species is assignable to the genus *Cohicaleyrodes* Bink in view of lobulate margin, absence of thoracic and caudal tracheal pores or clefts and folds, presence of cephalic, mesothoracic, eighth abdominal and caudal setae and elevated vasiform orifice. Hence a new combination *Cohicaleyrodes indicus* (David and Selvakumaran) is proposed here for *Mixaleyrodes indicus* David and Selvakumaran.

***Cohicaleyrodes jesudasani* (Jesudasan and David) nomen novum**

*Cohicaleyrodes indicus* Alexander Jesudasan and David, 1991: *Oriental Insects* **25**: 298–299. Host plant: *Sterculia alata*, Dehra Dun, 13.02.1985, coll. R. W. A. Jesudasan.

In 1991 Jesudasan and David described *Cohicaleyrodes indicus* from *Sterculia alata* collected at Dehra Dun on 13.02.1985. As *Mixaleyrodes indicus* has been assigned to the genus *Cohicaleyrodes* and pre-occupies *Cohicaleyrodes indicus* Jesudasan and David, the new name *Cohicaleyrodes jesudasani* is proposed here for *Cohicaleyrodes indicus* Jesudasan and David.

## REFERENCES

- Alexander Jesudasan, R. W. and David, B. V. (1991) Taxonomic studies on Indian Aleyrodidae (Insecta: Homoptera). *Oriental Insects* **25**: 231–434.
- David, B. V. and Selvakumaran, S. (1987) A new species of whitefly *Mixaleyrodes indicus* sp. nov. (Aleyrodidae: Homoptera) from India. *Journal of Bombay Natural History Society* **84**: 654–656.

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## **Influence of leaf preservation methods on seed crop rearing of silkworm *Bombyx mori* (L)**

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**ABSTRACT:** Different methods of leaf preservation were evaluated by analyzing the influence on some of the important traits. Maximum values for most of the parameters like chawki loss, larval weight, cocoon weight, shell weight and ERR were recorded in batches receiving leaves stored as twigs and minimum for those receiving leaf stored in basket, irrespective of the season. However, during summer, leaves preserved in polythene covers gave lowest values for various characters studied. Leaves preserved on twigs have better nutritive value than others.

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**KEYWORDS:** leaf preservation; seed crop rearing; economic traits

Leaf quality is affected considerably between harvesting and feeding to the mulberry silkworm. During preservation of leaf, moisture decreases (Yokoyama, 1962), while protein and starch gets decomposed into amino acids and sugars respectively (Rangaswamy *et al.*, 1976). Moisture retention capacity of leaf during preservation depends upon the method of leaf preservation, environmental conditions, time of harvest and duration of preservation, besides variety. The microclimate during leaf preservation and in the rearing bed also influence the leaf quality and its acceptability to the silkworm. Different methods have been suggested for preservation of leaf. Rashid *et al.* (1996) have shown that leaf preserved in gunny cloth was better than that preserved in plastic bag. Leaf stored in wooden framed boxes covered with wet gunny cloth was found to be suitable for better cocoon production (Kasiviswanathan *et al.*, 1973; Krishnaswami, 1978; Sarkar *et al.*, 1989). The quality of mulberry leaves also differs depending upon the maturity of leaves (Ito and Kobayashi, 1978). Narasimhamurthy *et al.* (1987) have reported that the time of harvesting also affected the quality of mulberry leaves. Due to active photosynthesis and transpiration, leaves harvested late afternoon contain less moisture and more carbohydrates and wither fast compared to early morning harvested leaves (Sarkar *et al.*, 1989). In this paper comparative evalu-

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ation of different methods of leaf preservation and its influence on seed crop rearing is presented.

Individual leaves and twigs with leaves harvested from the irrigated M5 garden maintained as per norms were preserved by different methods. The leaves and twigs were harvested twice daily, once during morning and evening, during cooler hours. Four feeding systems adopted with the feed being offered at 6 am, 10 am, 4 pm and 10 pm. The different methods of preservation adopted included (1) Leaf chamber method: leaves were preserved in the chamber covered on all sides with wet gunny cloth (Krishnaswami *et al.*, 1973), (2) Mud pot method: leaves were preserved in the pot covered with wet gunny cloth (Krishnaswami *et al.*, 1973), (3) Polythene cover method: leaves were kept in polythene cover, (4) Twigs method: the twigs with leaves kept in chamber was covered with wet gunny cloth (Sekharappa *et al.*, 1997), (5) Basket method: leaves were kept in the basket without any covering. Preservation adopting the above approaches was restricted for a maximum period of 12 h.

A bivoltine race NB4D2 spinning dumbbell cocoons was used for the studies. Rearing of NB4D2 was done in cellular fashion with minimum of three replications per treatment and was conducted as per the method of Krishnaswami (1978). Data on chawki loss, V instar maximum larval weight, cocoon weight, shell weight and effective rate of rearing (ERR) were collected and statistically analyzed. After the cocoons were harvested, uniform size cocoons for each treatment were retained and layings were prepared. The total number of eggs deposited by individual moths was recorded for all the treatments. Data for twenty moths per each treatment have been assessed for statistical significance to assess the influence of leaf preservation methods on fecundity.

The minimum chawki loss of 6.2% was recorded during rainy season for leaves preserved on twigs (Table 1). This was followed by leaves preserved in mud pot which recorded a chawki loss of 10.2%. Maximum loss of 24.6% was recorded for leaves preserved in basket. During winter, there was no significant effect of different methods of leaf preservation on chawki loss. However, during summer, a trend similar to rainy season was observed, with leaves preserved on twigs performing better than leaf from other sources.

The maximum weight for 10 larvae recorded was 43.7 g for larvae reared on leaf preserved on twigs and the minimum weight of 38.4 g was recorded for leaf preserved in basket (Table 1). The larvae reared on leaves preserved in mud pot weighed 41.9 g. During winter, similar to that recorded in rainy season, the maximum weight was recorded for larvae reared on twig source leaf (42.3 g) followed by batches receiving leaves preserved in mud pot (40.1 g) and minimum for those reared on polythene cover. During summer minimum growth was recorded for batches reared on leaves covered with polythene (36.5 g) and maximum for those reared on forleaf twigs (39.5 g).

Maximum ERR (97%) was recorded in batches receiving leaves from twigs and minimum of 85% for those reared on basket. Larvae reared on mud pot leaf has yielded nearly 94% cocoons and the controls about 90%. The yield during winter for the batches receiving leaves mulberry preserved as twigs, in baskets, in mud pot

TABLE 1. Effect of different methods of leaf preservation on growth performance of silkworm *Bombyx mori*

| Season | Leaf treatment  | Chawki loss (%) | Wt. of 10 larvae (g) | Yld/10,000 larvae (g) | Cocoon wt (g) | Shell wt (g) | Fecundity* (no.) |
|--------|-----------------|-----------------|----------------------|-----------------------|---------------|--------------|------------------|
| Rainy  | Leaf chamber    | 14.72           | 40.40                | 9010                  | 1.70          | 0.34         | 530              |
|        | Mud pot         | 10.2            | 41.93                | 9350                  | 1.74          | 0.35         | 548              |
|        | Polythene cover | 13.72           | 38.50                | 8600                  | 1.64          | 0.32         | 492              |
|        | Twigs           | 6.22            | 43.66                | 9683                  | 1.78          | 0.36         | 601              |
|        | Basket          | 24.64           | 38.37                | 8450                  | 1.41          | 0.29         | 458              |
|        | CD at 5%        | 2.49            | 1.22                 | 337.68                | 0.02          | 0.01         | 42.28            |
| Winter | Leaf chamber    | 11.96           | 38.17                | 9133                  | 1.61          | 0.33         | 524              |
|        | Mud pot         | 12.15           | 40.10                | 9233                  | 1.71          | 0.34         | 527              |
|        | Polythene cover | 10.20           | 30.93                | 9083                  | 1.63          | 0.30         | 423              |
|        | Twigs           | 11.91           | 42.26                | 9566                  | 1.74          | 0.35         | 541              |
|        | Basket          | 12.78           | 37.50                | 8450                  | 1.63          | 0.30         | 395              |
|        | CD at 5%        | —               | 0.88                 | 479.245               | 0.038         | 0.012        | 32.723           |
| Summer | Leaf chamber    | 17.56           | 37.13                | 8433                  | 1.45          | 0.30         | 517              |
|        | Mud pot         | 13.80           | 37.83                | 8933                  | 1.48          | 0.31         | 528              |
|        | Polythene cover | 24.94           | 36.60                | 8883                  | 1.46          | 0.30         | 532              |
|        | Twigs           | 9.71            | 39.46                | 9600                  | 1.53          | 0.32         | 591              |
|        | Basket          | 19.16           | 33.70                | 7216                  | 1.35          | 0.26         | 462              |
|        | CD at 5%        | 5.13            | 1.761                | 689.039               | 0.036         | 0.011        | 19.961           |

\*Subsequent generation

and leaf chamber (control) were 96, 85, 92 and 91%, respectively. During summer the yield was more or less similar to that recorded during other seasons.

The single cocoon weight during rainy season was in the order of 1.78, 1.70, 1.70, 1.62 and 1.41 g for larvae reared on leaves preserved on twigs, in mud pot, leaf chamber, polythene cover, and basket respectively. During winter, maximum cocoon weight of 1.74 g was recorded for leaves on twigs. During summer, maximum cocoon weight was obtained for leaves on twigs and minimum for leaves in basket.

For the trait shell weight, Maximum shell weight of 0.36, 0.35 and 0.32 g was recorded during rainy, winter and summer months respectively, for insects reared on twigs. Lowest shell weight, of 0.29, 0.30 and 0.26 g were recorded for insects reared on leaves preserved in basket, during rainy, winter and summer respectively. For the other treatments the shell weight did not differ markedly irrespective of the season.

Similar observations were recorded for fecundity, with maximum for insects reared on twigs and minimum was recorded for those on leaves in basket (Table 1).

From this study it is clear that leaf quality is influenced by the method of preservation. Among various factors, the quality of mulberry leaves contribute maximum towards rearing performance (Vineethkumar *et al.*, 1995; Mathur *et al.*, 1995). Kasiviswanathan *et al.* (1973) reported that considerable water is lost during storing affecting feeding and cocoon production. Feeding on leaves stored for a long period prolonged the larval duration, decreased body weight, cocoon formation,

cocoon weight, and shell ratio (Krishnaswami *et al.*, 1970). Preserving the leaf for prolonged periods results in utilization of carbohydrates for respiration and water content for transpiration (Sengupta *et al.*, 1971). The leaf moisture retention capacity has positive effect on the nutritive value of the leaf in general and ingestion and digestibility in particular. Thus it is of utmost importance to minimize the water loss from the leaves, after harvesting. The ideal temperature for preservation of leaf is about 20°C and relative humidity is about 90% (Rangaswamy *et al.*, 1976).

Various methods are adopted in the industry for leaf preservation. Vineethkumar *et al.* (1995) observed that leaf preservation in polythene bags reduces the quality of leaf due to increased humidity coupled with rise in temperature and lack of aeration. Sarkar *et al.* (1989) showed that leaf preservation in framed box covered with gunny cloth and in polythene bag are most suitable for silkworm. Krishnaswami *et al.* (1970, 1973) suggested preservation of leaf in chambers covered with wet gunny cloth.

Influence of five different methods of leaf preservation covering different seasons on rearing performance indicate, minimum chawki loss when the leaves were preserved on twigs and maximum when the leaves were preserved in basket. The growth of the larvae is minimum during rainy and winter seasons for batches receiving leaves preserved in basket, while maximum weight has been achieved when leaves on twigs are provided. During summer least weight is recorded for those fed with leaves covered with polythene bag. The chawki loss, larval growth, cocoon yield, cocoon and shell weight indicated best performance on leaves preserved on twigs, irrespective of the season. Leaf preserved in basket was inferior to that preserved in polythene cover, irrespective of season. The preservation of leaf in chamber covered with wet gunny cloth or in mud pot covered with wet gunny cloth were better than basket and polythene cover treatments.

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#### REFERENCES

- Ito, T. and Kobayashi, M. (1978) Rearing of silkworm. In: *Silkworm: An Important Laboratory Tool*, Tazima, Y (Ed). Kodansha Ltd: Tokyo, Japan, p. 84.
- Kasiviswanathan, K., Krishnaswami, S. and Venkataram, C. V. (1973) Effect of storage on the moisture content of mulberry leaves. *Indian J. Seric.* **12**: 3–21.
- Krishnaswami, S., Ahsan, M and Shridharan, T. P. (1970) Studies on the quality of mulberry leaves and silkworm cocoon crop production. *Indian J. Seric.* **9**: 11–25.
- Krishnaswami, S., Narasimhanna, M. N., Suryanarayana, S. K. and Kumarraja, S. (1973) Sericulture manual–2. Silkworm rearing. In: *Food and Agricultural Organization*. United Nations: Rome, 53–90.
- Krishnaswami, S. (1978) New technology of Silkworm rearing, CSR TI. Bulletin no 2.
- Mathur, V. B., Singh, G. P., Himantharaju, M. T., Kamble, C. K. and Datta, R. K. (1995) Evaluation of leaf preservation methods for chawki rearing and its impact on cocoon crop in Silkworm *Bombyx mori*. *L. Bull. Seric. Res. Bangladesh* **6**(2): 111–115.

- Narasimhamurthy, C. V., Donatus, E. and Pillai, S. V. (1987) Chemical composition of Mulberry during preservation and leaf storage. *Sercologia* **27**(2): 623–627.
- Rangaswamy, G., Narasimhanna, M. N., Kasiviswanathan., Shastry, C. R. and Jolly, M. S. (1976) Seri cultural manual–1. Mulberry cultivation FAO. In: *Agricultural Services Bulletin 15/1*, Food and Agricultural Organization of United Nations: Rome, 65–91.
- Rashid, M. A., Mahadevamurthy, T. S., Shivakumar, G. R. and Magadum, S. B. (1996) Studies on effect of preservation of bush and tree leaf on economic parameters of silkworm, *Bombyx mori*. *L. Bull. Seric. Res.* **7**: 61–63.
- Sarkar, A. A., Quader, M. A. and Ahmed, S. V. (1989) Effect of types and duration of storage of moisture content in mulberry leaves. *Bangladesh J. Agric.* **14**(1): 37–44.
- Sengupta, K., Sing, B. D. and Mustodi, J. C. (1971) Study on the effect of harvest of mulberry leaves on silkworm *Bombyx mori*, L. on cocoon crop quality. *Indian J. Seric.* **10**(1): 1–5.
- Sekharappa, B. M., Gururaj, C. S., Raghuraman, R. and Dandin, S. B. (1997) Shoot Feeding for late age Silkworm. *KSSRDI. Publication: 1–27*.
- Vineethkumar, Himantharaj, M. T., Rajan, R. K., Singh, G. D., Mathur, V. B., Kamble, C. K. and Datta, R. K. (1995) Study on the effect of mulberry leaf preservation and its impact on cocoon crop and cocoon quality in silkworm *Bombyx mori*. *L. Uttarpradesh J. Zool.* **14**(14): 65–69.
- Yokoyama, T. (1962) Synthesised science of sericulture. Published by CSB, Bombay, India pp. 1–306.

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## A report on two new and two unrecorded species of *Drosophila* from Mukteshwar, Kumaon, India (Diptera: Drosophilidae)

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**ABSTRACT:** Taxonomic description of two new species viz., *Drosophila mukteshwarensis* and *Drosophila sargakhetensis* and new distribution records of *Drosophila hubeiensis* Sperlich and Watabe, 1997 and *Amiota pseudotau* Toda and Peng, 1990 is given. A list of Drosophilidae collected from Mukteshwar is also provided.

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**KEYWORDS:** *Drosophila*, Drosophilidae, new species, new records, Kumaon, India

The first use of the family name Drosophilidae was probably by the German dipterist, Hermann Lowe, in several papers published in 1862. The family contains nearly 3600 species around the world (Wheeler, 1981, 1987 and unpublished, Bachli, 1998). The members of the family Drosophilidae particularly the genus *Drosophila* occupy very important position among the organisms that are used as material for genetics studies. The Indian Drosophilid fauna has been studied extensively in recent years and most areas have been covered in collections (Gupta (1969, 1970, 1971, 1972); Gupta and Ray-Chaudhuri (1970a,c); Gupta and Singh (1977, 1979); Singh and Gupta (1977a,b, 1981); De and Gupta (1996a,b); Reddy and Krishnamurthy (1968, 1970)).

Mukteshwar (29° 28'N lat. and 79° 39' E long.) in Kumaon region, a hilly area is located at an elevation of about 2250 meter (7,500 feet) amsl. The area is characterized by having dense evergreen Oak forest (*Quercus floribunda*) with medium to steep slopes. The forest vegetation consists of broad leaved and coniferous species and extremely moist condition due to heavy rainfall. Although some of the areas have been surveyed and have yielded very interesting results (Singh and Bhatt, 1988; Singh and Negi, 1989, 1992, 1995, Singh *et al.*, 2000, 2004; Fartyal and Singh, 2004), still a vast area of Kumaon region is unknown for its Drosophilid fauna. Looking into the

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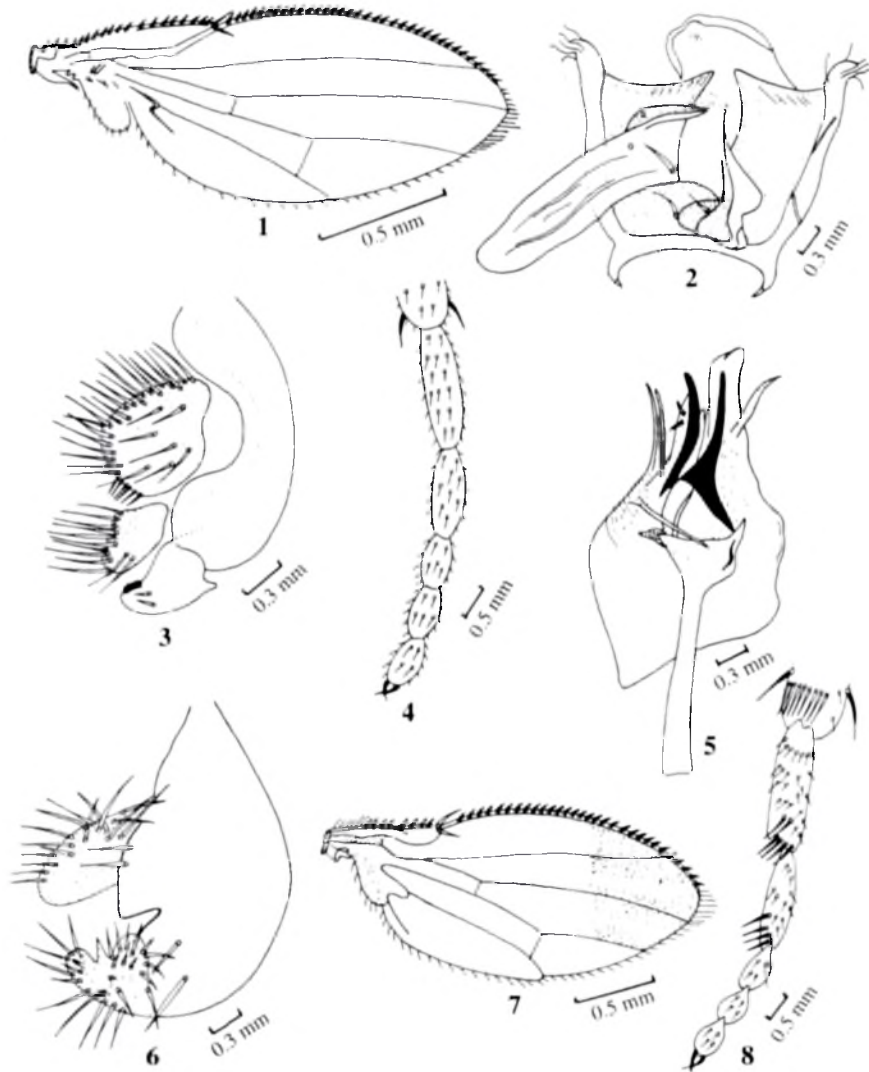


FIGURE 1. (1)–(4): *Drosophila muktesharensis* sp. nov. (1) wing; (2) phallic organs; (3) periphallalic organs; (4) foreleg (♂). (5)–(8): *Drosophila sargakhetensis* sp. nov. (5) phallic organs; (6) periphallalic organs; (7) wing; (8) foreleg (♂).

above situation Drosophilidae of Mukteshwar was surveyed which has yielded two new species and two new records from this region.

The Drosophilid flies were collected at several collecting stations in Mukteshwar. Following two methods were employed to collect Drosophilid flies. (1) Trap- bait and (2) Net-sweeping methods.

The collected flies were etherized, categorized and the species were identified under Wild-Leitz- Stereozoom Microscope. The males were studied as such but the individual females, which could not be identified, were isolated and allowed to breed in separate vials containing standard laboratory food medium. The progeny obtained from such single gravid females were used for species identification. Camera Lucida drawings of taxonomic characters of the species were made.

In general, descriptions of different species were based on adult specimens. The terminology used in the descriptions of *Drosophila* species, periphallallic and phallic organs is as proposed by Sturtevant (1942), Hsu (1949) and McAlpine (1981) respectively.

### **Genus *Drosophila* Fallén**

*Drosophila* Fallén, 1823. p. 4. Type species *Musca funebris* Fabricious, 1787, by subsequent designation (Zetterstedt), type locality, Europe.

#### *Diagnosis*

Arista usually plumose (exceptionally with reduced rays, never micropubescent), anterior reclinate orbital setae small, postvertical setae well developed, scutellum typically with 6 or more rows of acrostichal setulae and two pairs of dorsoventral setae, prescutellar setae enlarged or not, sternopleuron with up to 3 microchaetae.

#### *Subgenus Drosophila Fallén S. Str.*

Subgenus *Drosophila* Fallén S. Str. *Drosophila* Fallen, Diptera, Sveciae Geomyz. 2:4 Type species: *Musca funebris* Fabricious.

#### *Diagnosis*

Gena often broad: subvibrissal setae large, prescutellar setae not or barely, enlarged; propleural bristles absent, apical bands on anterior abdominal tergites when present, usually interrupted in the middle. Usually rather large species.

#### *Drosophila (Drosophila) mukteshwarensis sp. nov*

Average length of body: 3.60 mm ( $\sigma$ ).

#### *Diagnosis*

Aedeagus long and broad, aedeagal dorsal process small, ca.7. prenisetae arranged in nearly straight or slightly concave row on surstylus.

*Head, ♂*

Arista with about 3 upper and 2 lower branches in addition to terminal bifurcation. Antenna with pedicel and flagellomere grayish brown. Frons including the ocellar triangle blackish brown. Orbitals in the ratio 11 : 8 : 16. Facial carina brownish yellow. Palpus light brown, vibrissa prominent and subvibrissal setae equal. Gena yellow, greatest width of gena 0.16 the greatest diameter of eye. Eyes bright red.

*Thorax ♂*

Brown in colour with light yellow linings. Acrostichal setulae regular in 8 rows. Anterior scutellars convergent and crossing each other, posterior parallel. Scutum light brown and scutellum gray with 4 yellow patches. Ventral part of scutellum dark brown. Sterno-index 0.81. Thoracic pleura light brown. Legs dark brown. Preapicals on all three tibiae, apicals on first and second tibiae [Fig. 1(4)].

*Wings, ♂ [Fig. 1(1)]*

Wing clear, posterior cross (dM-Cu) vein slightly fouscous. C<sub>1</sub> setulae two, equal, C<sub>3</sub> fringe 0.50. Average wing vein indices C-index 3.60; 4V-index 1.61; 4C-index 0.71; 5X-index 1.67. Haltere stem brown and knob pale yellow.

*Abdomen, ♂*

Abdominal tergites yellowish brown. 1st, 2nd and 3rd abdominal tergites with light brown bands and 4th, 5th and 6th abdominal tergites with dark brown bands in male interrupted in the middle, 6th not interrupted.

*Male terminalia, (Periphallic organs, Fig. 1(3))*

Epandrium brown not pubescent; cardoventrally broad and inner surface of epandrium concave. Epandrium with one ventral lobe with about 11–12 hair or spines. Surstylus with about 6–7 prenisetae arranged in nearly straight or slightly concave row. Cercus oval pubescent with 7 small and stout hair and about 28 long setae.

*Phallic Organs, [Fig. 1(2)]*

Aedeagus long and slender, basal apodeme half the length of aedeagus. Parameres leaf like with two apical sensilla. Gonopod fused. Hypandrium somewhat quadrate and ventrally slightly tapering, upper caudo dorsal margin with several fine hair.

*Holotype, ♂*

India: Uttaranchal, Kumaon, Nainital district, Mukteshwar, 26 August 2000 coll. Joshi (DZKU).

*Paratype, 08♂*

Same data as the holotype. Deposited in the department of Zoology, Kumaon University, Nainital.

*Distribution*

India (Uttaranchal, Kumaon, Nainital district, Mukteshwar).

*Relationship*

This species closely resembles *D. curviceps* Okada and Kurokawa in the structure of its aedeagus but distinctly differs from it in many other details.

*Etymology*

In reference to the type locality of this species.

**Subgenus *Sophophora* Sturtevant**

*Sophophora* Sturtevant, 1939, *Proc. Nat. Acad. Sci.*, **25**: 139. Type species *Drosophila melanogaster* Meigen, 1830; type locality: Austria and Germany.

*Diagnosis*

Apical bands on anterior abdominal tergites, when present, not interrupted in the middle; subvibrissal setae relatively large; gena usually relatively narrow; prescutellar setae absent; propleural setae absent.

***Drosophila sargakhetensis* sp. nov.**

Average length of the body: 2.6 mm (♂).

*Diagnosis*

Antenna, upper half of frons, clypeus and palpus blackish; thoracic pleura, fore coxa, femur and abdominal tergites dark brown; lower half of frons milky white; aedeagal basal process not serrated at least on distal half margin.

*Description*

## Head, ♂

Arista with 4 dorsal and 3 ventral branches in addition to terminal bifurcation. Antenna with pedicel and flagellomere blackish. Frons including the ocellar triangle light yellowish brown. Orbitals in the ratio 9 : 6 : 13. Facial carina yellow. Palpus blackish brown. Clypeus blackish brown in colour. Vibrissa long and subvibrissal setae equal. Gena dark brown, greatest width of gena 0.15 the greatest diameter of eye. Eyes dark red.

*Thorax, ♂*

Scutum and scutellum light brown. Acrostichal setulae regular in 8 rows. Anterior scutellars convergent and crossing each other, posterior slightly divergent. Sterno-index 0.71. Legs brown. 1st and 2nd tarsomere of foreleg with sex spines, 1st tarsomere with 4 + 2 spines and 2nd tarsomere with 3 + 1 sex spines [Fig. 1(8)].

*Wings, ♂ (Figure 1(7))*

Presence of black patches at the tip of  $R_{2+3}$  and  $R_{4+5}$  wing veins;  $R_{2+3}$  slightly curved to costa at tip;  $R_{4+5}$  and  $M$  distally slightly convergent  $C_1$  setulae two unequal;  $C_3$  fringe 0.60. Average wing vein indices: C-index 2.41; 4V-index 2.00; 4C-index 1.09; 5X-index 2.00. Haltere stem and knob light brown in colour.

*Abdomen, ♂*

In male 1st to 4th tergites light brown; 5th and 6th tergites dark black.

*Male terminalia (Periphallic organs, Figure 1(6))*

Epandrium broad, bare with about 4–5 long bristle. Surstylus somewhat triangular, pubescent with about 6 small setae and 20–22 long setae. Cercus oval and pubescent with about 17–18 long setae.

*Phallic organs [Fig. 1(3)]*

Aedeagus slender and bifid. Basal apodeme of aedeagus double length of aedeagus. Anterior parameres large, black and apically pointed. Posterior parameres small and leaf like with two apical sensilla. Hypandrium bilobed. Ventral fragma longer than broad.

*Holotype, ♂*

India, Uttaranchal, Kumaon, Nainital districts, Sargakhet, 2 July 1999, coll. Joshi (DZKU).

*Paratype, 2♂*

Same data as holotype. Deposited in the Department of Zoology, Kumaon University, Nainital.

*Distribution*

India (Uttaranchal, Kumaon, Nainital district, Sargakhet).

*Relationship*

This species is a member of the subgenus *Sophophora* of the genus *Drosophila* where it resembles *D. nepalensis* Okada, 1955 in the structure of wings and other morphological details but distinctly differ from it in genital structures.

*Etymology*

Patronym referring to the type locality.

***Drosophila hubeiensis* Sperlich and Watabe**

*Drosophila hubeiensis* : Sperlich and Watabe, 1997, *Jpn. J. Ent.*, **65(3)**: 621–633.

Taxonomic characters: As described by Sperlich and Watabe, 1997.

*Specimens examined*

India: Uttaranchal, Kumaon, Nainital district, Bhatelia, 02, ♂ and 02♀ 23 July, 1999, coll. Joshi. July, 1999, coll. Joshi.

*Distribution*

Highlands of Hubei and Sichuan Provinces, Central China, North India (Kumaon, Mukteshwar, new locality).

**Subgenus-*Phortica* Schiner**

*Phortica* Schiner, 1862, wien. ent. Monatschr; 6:433; Okada, 1997, Kontyu, 39:96; MACA, 1977, Acta ent. bohemoslov., 74:116. Type species *Drosophila verigata* FALLEN, 1823.

***Amiota (Phortica) pseudotau* Toda and Peng**

*Amiota pseudotau* Toda and Peng, 1990. Entomotoxonomia Vol. XII(1): 41–55.

Taxonomic characters: As described by Toda and Peng, 1990.

*Specimens examined*

India: Uttaranchal, Kumaon, Nainital district, Mukteshwar 1♂, 13 August 2000, coll. Joshi.

*Distribution*

China, Gangdong; India (Kumaon, Mukteshwar, new locality).

**List of *Drosophilidae* collected from Mukteshwar:**

| Genus/Subgenus                    | Species   |
|-----------------------------------|---|
| Genus- <i>Amiota</i> Lowe         | 1. <i>bandes</i> Singh and Negi (1992)                              |
|                                   | 2. <i>biprotrusa</i> Chen and Toda, 1998                            |
| Subgenus- <i>Phortica</i> Schiner | 3. <i>pseudotau</i> Toda and Peng, 1990<br>(new record, this paper) |
| Genus- <i>Dettopsomyia</i> Lamb   | 4. <i>nigrovittata</i> (Malloch, 1924)                              |

1d Toda,

**Genus- *Drosophila* Fallen****Subgenus- *Drosophila* Sturtevant**

5. *analspina* Singh and Negi (1995)
6. *bishtii* Singh and Negi (1995)
7. *bizonata* Kikkawa and Peng, 1938
8. *immigrans* Sturtevant, 1921
9. *lacertosa* Okada (1956)
10. *mukteshwarensis* (new species, this paper)
11. *nainitalensis* Singh and Bhatt (1988)
12. *notostriata* Okada, 1966
13. *Painai* Singh and Negi, 1995
14. *parazonata* Dwivedi and Gupta, 1980
15. *repleta* Wollaston, 1858
16. *sulfurigaster* Duda, 1923
17. *trizonata* Okada, 1966

**Subgenus- *Sophophora* Sturtevant**

18. *bifasciata* Pomini, 1940
19. *hubiensis* Sperlich and Watabe, 1997  
(new record, this paper)
20. *jumbulina* Parshad and Paika, 1964
21. *melanogaster* Meigen, 1930
22. *kikkawai* Burla, 1954
23. *nepalensis* Okada, 1955
24. *sarswati* Singh and Dash, 1995
25. *sargakhetensis* (new species, this paper)
26. *suzukii indicus* Parshad and Paika, 1964
27. *takahashii* Sturtevant, 1927

**Genus- *Gitona* Meigen****Genus- *Hirtodrosophila* Duda**

28. *distigma* Meigen, 1830
29. *hexaspina* Fartyal and Singh, 2000
30. *quadrivittata* Okada, 1956

**Genus- *Leucophenga* Mik**

31. *albiceps* de Meijere, 1914
32. *angulata* Singh, Dash and Fartyal, 2000
33. *angusta* Okada, 1956
34. *bellula* (Bergroth, 1894)
35. *clubiata* Singh, Dash and Fartyal, 2000
36. *neolacteusa* Singh and Bhatt, 1988
37. *ornata* Wheeler, 1959
38. *subpollinosa* (de Meijere, 1914)

**Genus- *Lissocephala* Malloch****Genus- *Paraleucophenga* Hendel**

39. *parasiatica* Takada and Momma, 1975
40. *neojavanaii* Singh and Negi, 1992

**Genus- *Scaptomyza* Hardy**

41. *elmoi* Takada, 1970
42. *quadruangulata* Singh and Dash, 1993

- Bachli, G. (1998) Family Drosophilidae In: contributions to a Manual of Palaeartic, Diptera. *Higher Brachycera*. Papp, L. and Darvas, B. (Eds). Budapest, vol. 3: 503–514.
- De, A. and Gupta, J. P. (1996a) Records of Drosophilid species from Bhutan (Diptera: Drosophilidae). *Drosophila. Information Service* 77: 98.
- De, A. and Gupta, J. P. (1996b) Records of Drosophilid species from West Bengal with description of one new and two previously recorded species from India (Insecta: Diptera: Drosophilidae). *Senckenbergiana Biologica* 76(1/2): 129–133.
- Fartaly, R. S. and Singh, B. K. (2002) Taxonomic accounts of new species of Drosophilidae (Insecta: Diptera) of Kumaon region, India. *Entomon* 27(4): 355–364.
- Fartaly, R. S. and Singh, B. K. (2004) A new species of genus *Paraleucophenga* (Diptera: Drosophilidae) from Kumaon region. *India, Senckenbergiana Biologica* 83(2): 177–180.
- Gupta, J. P. and Ray-Chaudhuri, S. P. (1970a) Drosophilidae of Chakia forest, Varanasi, India. *Drosophila. Information Service* 45: 168.
- Gupta, J. P. and Ray-Chaudhuri, S. P. (1970c) Some new and unrecorded species of *Drosophila* (Diptera: Drosophilidae) from India. *Proceeding of Royal Entomological Society London* (B) 39: 57–72.
- Gupta, J. P. and Singh, B. K. (1979) Two new species of *Drosophila* from Shillong, Meghalaya (Diptera: Drosophilidae). *Entomon* 4(2): 167–172.
- Gupta, J. P. and Singh, B. K. (1977) Two new and two unrecorded Indian species of *Drosophila* from Kurseong, Darjeeling. *Ent. Month. Mag. Oxford* 113: 71–78.
- Gupta, J. P. (1969) A new species of *Drosophila* Fallen (Insecta: Diptera: Drosophilidae) from India. *Proceeding of Zoological Society, Calcutta* 22: 53–62.
- Gupta, J. P. (1970) Description of a new species of *Phorticella*, *Zaprionus* (Drosophilidae) from India. *Proc. India. Nat. Sci. Acad. B* 36: 62–70.
- Gupta, J. P. (1971) A new species of *Drosophila* (*Scaptodrosophila*) from Varanasi, India. *Amer. Midl. Naturalist* 86(2): 493–496.
- Gupta, J. P. (1972) *Drosophila orrisaensis*, a new species of *Drosophila* from Orissa. *Oriental Insects* 6(4): 491–494.
- Hsu, T. C. (1949) The external genital apparatus of male Drosophilidae in relation to systematics. *University of Texas Publications* 4920: 80–142.
- McAlpine, J. F. (1981) Morphology and terminology-adults. In: *Manual of Nearctic Diptera, Agriculture, Canada Monograph No. 27*, McAlpine *et al.*, J. F. (Ed). Biosystematic Research Institute: Ottawa, Ontario, vol 1: 9–63.
- Okada, T. (1956) Systematic study of Drosophilidae and allied families of Japan. *Kontyu* 32: 105–115.

- Reddy, G. S. and Krishnamurthy, N. B. (1968) *Drosophila rajasekari* a new species from Mysore (India). *Proc. Indian Acad. Sci.* **68**: 202–205.
- Reddy, G. S. and Krishnamurthy, N. B. (1970) *Drosophila mysorensis* a new species of *Drosophila* (Diptera: Drosophilidae) from Mysore. *South India. J. Biol. Sci.* **13**: 24–29.
- Singh, B. K. and Gupta, J. P. (1977a) The subgenus *Drosophila* (*Scaptodrosophila*) In India (Diptera: Drosophilidae). *Oriental Insects* **11**(2): 237–241.
- Singh, B. K. and Gupta, J. P. (1981) Report on two new and three other newly recorded species of *Drosophila* from India (Diptera: Drosophilidae). *Studies in Natural Sciences* **2**(13): 1–8.
- Singh, B. K. and Bhatt, M. (1988) The Drosophilidae of Kumaon region with description of two new species and three new record. *Oriental Insects* **22**: 147–161.
- Singh, B. K. and Dash, S. (1993) Drosophilidae of Uttarakhand region with the description of four new species (Insecta: Diptera). *Proceeding of Zoological Society, Calcutta* **46**(2): 3–140.
- Singh, B. K. and Dash, S. (1998) Drosophilidae of Kumaon region, India with the description of four new species (Insecta: Diptera). *Proceeding of Zoological Society, Calcutta* **51**(1): 49–56.
- Singh, B. K. and Fartyal, R. S. (2002) Family Drosophilidae (Insecta: Diptera) in Kumaon region, India, with description of one new species and three new records. *Proceeding of Zoological Society, Calcutta* **55**(1): 11–18.
- Singh, B. K. and Gupta, J. P. (1977b) Two new and two unrecorded species of genus *Drosophila* Fallen (Diptera: Drosophilidae) from Shilong, Meghalaya, India. *Proceeding of Zoological Society, Calcutta* **30**: 31–39.
- Singh, B. K. and Negi, N. S. (1989) Drosophilidae of Garhwal region with the description of one new species. *Proceeding of Zoological Society, Calcutta* **40**: 19–25.
- Singh, B. K. and Negi, N. S. (1992) Two new and one unrecorded, species of Drosophilidae from Uttarakhand, India (Insecta; Diptera) Senckenbergiana. *Biologica* **72**: 321–327.
- Singh, B. K. and Negi, N. S. (1995) Further addition to the Drosophilidae of Uttarakhand, India, (Diptera: Drosophilidae). *Entomologische Zeitschrift* **105**(21): 421–440.
- Singh, B. K., Dash, S. and Fartyal, R. S. (2000) Further addition to the genus *Leucophenga* from Kumaon, India (Insecta: Diptera). *Senckenbergiana Biologica* **80**(1/2): 149–154.
- Singh, B. K., Dash, S. and Fartyal, R. S. (2004) Revision of the subgenus *Drosophila* (*Drosophila*: Diptera: Drosophilidae) of Kumaon region, India with the description of eight new species. *Senckenbergiana biologica* **83**(2): 163–176.
- Sperlich, D. and Watabe, H. A. (1997) *Drosophila hubeiensis* (Diptera, Drosophilidae) a new species of the *Drosophila obscura* species-group from the Mainland of China. *Japanese Journal of Entomology* **65**(3): 621–633.
- Sturtevant, A. H. (1942) The classification of the genus *Drosophila*, with descriptions of 9 new species. *University of Texas Publications* **42**13: 5–15.
- Toda, M. J. and Peng, T. X. (1990) Eight new species of the subgenus *Phortica* (Diptera: Drosophilidae, *Amiota*) from Guangdong Province, Southern China. *Entomotaxonomia* **12**(1): 11–55.
- Wheeler, M. R. (1981) The Drosophilidae; A taxonomic over view. In: *The Genetics and Biology of Drosophila*, Ashburner, M., Carson, H. L. and Thompson, J. N., Jr (Eds). Academic Press London: **Vol. 3a**: 1–97.
- Wheeler, M. R. (1987) Drosophilidae. In: *Manual of Nearctic Diptera*, **Vol. 2**, McAlpine, J. F. *et al.* (Ed). 1011–1018. Research Branch, Agricultural Canada, Ottawa Agric. Can. Monograph. No. **28**: Vit. 675–1322

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## ***Planococcus citri* (Risso)-an additional mealybug vector of *Badnavirus* infecting black pepper (*Piper nigrum* L.) in India**

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**ABSTRACT:** Citrus mealybug (*Planococcus citri* (Risso), commonly found associated with black pepper (*Piper nigrum* L.) plants in India was shown to transmit *Badnavirus* associated with stunted disease on the basis of symptomatology and polymerase chain reaction using *Badnavirus* specific primers.

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**KEYWORDS:** *Badnavirus*, black pepper, citrus mealybug, *Piper nigrum*, *Planococcus citri*

Many species of mealybugs (Heteroptera: Pseudococcidae), besides being pests of many crops are also vectors of plant viruses, and are known to transmit many badnaviruses, closteroviruses and trichoviruses (Hull, 2002). The important genera involved in transmission of viruses include *Pseudococcus*, *Planococcus* and *Ferrisia* (Roivainen, 1980). Among them, *Planococcus* spp. and *Ferrisia virgata* (Cockerell) are commonly observed to infest black pepper (*Piper nigrum* L.) plants, the dried berries of which form an important item of international commerce for India, earning around Rs. 88 crores annually through export (Selvan, 2002). The crop is mainly grown in Kerala and Karnataka. However, stunted disease caused by viruses is an important production constraint of black pepper in India and other black pepper growing countries ((Duarte *et al.*, 2001; Lockhart *et al.*, 1997; Sarma *et al.*, 2001; Bhat *et al.*, 2003)). The disease is characterized by distortion, reduction in size, mottling and mosaic on leaves along with stunting of the whole plant, short spike length and poor filling of spikes leading to reduction in yield (Fig. 1). Two viruses namely, *Cucumber mosaic virus* (CMV) and *Badnavirus* were found associated with the disease (Sarma *et al.*, 2001; Bhat *et al.*, 2003). As black pepper is vegetatively propagated, the primary spread of the disease occurs through use of infected cuttings for planting and secondary spread in the field takes place through insect vectors. *Badnavirus* infecting

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FIGURE 1. A naturally infected black pepper plant showing typical symptoms of *Badnavirus* infection.

black pepper was shown to be transmitted by striped mealybug, *F. virgata* in India (Bhat *et al.*, 2003). This paper reports an additional vector, namely, citrus mealybug, *Planococcus citri* (Risso) involved in the transmission of *Badnavirus* infecting black pepper in India.

The *Badnavirus* infected black pepper isolate collected during October 2003 from Indian Institute of Spices Research (IISR), Experimental Farm, Peruvannamuzhi, and maintained by vegetative propagation under insect proof glasshouse conditions was used as source for transmission studies. Mealybug transmission tests were done using *P. citri* commonly seen on shoots of black pepper plants (Fig. 2). The adults were collected from black pepper plants in the field (from Wyanad District, Kerala) and reared on mature pumpkins in the laboratory. After three generations on pumpkin, the non-viruliferous young adult female mealybugs were given a 24 h acquisition access on symptomatic black pepper leaves (on the lower surface) kept in petri plates lined



FIGURE 2. Citrus mealybug, *Planococcus citri* used in the transmission tests.

with moist filter paper and covered with black cloth. Healthy black pepper seedlings were raised from seeds under insect-proof conditions inside the greenhouse. Fifteen mealybugs each were then transferred to 30 day old healthy test seedlings of black pepper (20 seedlings of cv. Karimunda and 10 seedlings of var. Panniyur-I) at four leaf stage kept in a cage covered with black cloth. After an inoculation access period of 24 h, the plants were sprayed with chlorpyrifos @ 0.075% and were then removed from the cages and kept for observation in the insect proof glasshouse maintained at about 28 °C.

To confirm the presence of virus in the plants exhibiting symptoms of virus disease, total nucleic acids extracted from the plants were subjected to polymerase chain reaction (PCR) using *Badnavirus* specific primers. The protocol of de Silva *et al.* (2002) was used for isolation of total nucleic acids from plants. The primer pair derived from the hypothetical protein gene sequence of *Badnavirus*, *Piper yellow mottle virus* (PYMV) (de Silva *et al.*, 2002) was used to prime the amplification. The genome sense primer 5' CTCCTTCATCTCCTCAAGAAGCCT 3' was derived from the beginning of the first 24 bases of the coding region. The genome antisense primer, 5' CACCCCGGGCCAAAGCTCTGATACCA 3' represented the last 27 bases of the coding region of the hypothetical protein gene (Gen Bank accession number AJ626981). The PCR reaction (50 µl) contained 200 ng each of the primer, 2.5 units Taq polymerase (Genei, Bangalore), 1×PCR buffer (Genei, Bangalore), and 10 µM each of the dNTPs (Finnzymes OY, Finland). PCR mix (40 µl) containing the above components was added to the tubes containing the template DNA (10 µl) resulting in a final reaction volume of 50 µl. Amplification was performed in an automated thermal cycler (Eppendorf Master Cycler Gradient) programmed for 5 cycles of 94°C for 30 sec, 37°C for 30 sec, and 72°C for 2 min, followed by 25 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 2 min. Following PCR, reaction products



FIGURE 3. *Planococcus citri* transmitted black pepper seedling exhibiting initial symptoms of the disease.

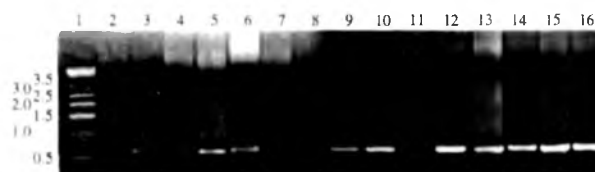


FIGURE 4. Agarose gel electrophoresis of polymerase chain reaction (PCR) products. Lane 1: 500 bp Marker DNA ladder; Lane 2: PCR product from uninoculated black pepper plant (negative control); Lanes 3–15: PCR product from mealybug transmitted black pepper plants; Lane 16: PCR product from badnavirus infected black pepper (positive control). Numbers on the left indicate MW of marker DNA bands in kb.

(20 $\mu$ l) were analysed by 1% agarose gel electrophoresis in Tris-acetate EDTA (TAE) buffer containing ethidium bromide. DNA was visualized and photographed using a UV transilluminator and a gel documentation apparatus (Alpha Innotech Corporation, USA). 500 bp ladder (Genei, Bangalore) was used as a size standard.

The *Badnavirus* could easily be transmitted by *P. citri* from naturally diseased black pepper to healthy seedlings of both cv. Karimunda and var. Panniyur-I. The initial symptoms of the disease like vein clearing and chlorotic mottle could be seen 4 weeks after inoculation (Fig. 3). Six out of 10 seedlings of Panniyur-I and 13 out of 20 seedlings of cv. Karimunda showed symptoms of the disease. The symptoms were more prominent on cv. Karimunda compared to var. Panniyur-I. Under natural

conditions also, disease symptoms are more prominent and severe on cv. Karimunda and related accessions while var. Panniyur-I shows mild symptoms. Total nucleic acid extracted from these plants when subjected to PCR, gave an expected PCR product of about 700 bp thus confirming the presence of virus in the plants (Fig. 4). No such band was seen in healthy seedlings. The specificity of the 700 bp band was confirmed by cloning and sequencing (data not shown).

*Badnavirus* is known to be associated with black pepper in many South East Asian countries and Brazil (Lockhart *et al.*, 1997; Duarte *et al.*, 2001; de Silva *et al.*, 2002; Bhat *et al.*, 2003). The disease was shown to be transmitted by *P. citri* in Indonesia, Philippines, Sri Lanka and Thailand (Lockhart *et al.*, 1997; de Silva *et al.*, 2002), *Pseudococcus elisae* in Brazil (Duarte *et al.*, 2001) and *F. virgata* in India (Bhat *et al.*, 2003). Thus, *P. citri* reported in this paper is an additional vector for the transmission of *Badnavirus* infecting black pepper in India. Nine species of mealybugs have been reported to be associated with black pepper in India (Devasahayam, 2000). Among them, *F. virgata*, *P. citri* and an undescribed *Planococcus* sp. are very common and abundantly seen. *F. virgata* and *P. citri* generally infest foliage and shoots while the undescribed *Planococcus* sp. exclusively infests roots.

Badnaviruses have been reported banana from (Anonymous, 1995), citrus (Ahlawat *et al.*, 1996), rice (Dasgupta *et al.*, 1996) and sugarcane (Viswanathan *et al.*, 1996) from India. In India, black pepper is grown as a mixed crop along with crops like banana, coffee, cocoa, citrus and tea, especially in Kerala and Karnataka. *P. citri* is also known to infest all these crops except tea and its main hosts include coffee, citrus and cocoa. These crops are grown to a large extent in Wyanad in Kerala where the incidence of viral disease on black pepper is also high (Bhat *et al.*, 2005). The present study suggests that *P. citri* found on these crops can also act as vector for the transmission of *Badnavirus* in black pepper.

## REFERENCES

- Ahlawat, Y. S., Pant, R. P., Lockhart, B. E. L., Srivastava, M., Chakraborty, N. N. and Varma, A. (1996) Association of badnavirus with citrus mosaic disease in India. *Plant Disease* **80**: 590–592.
- Bhat, A. I., Devasahayam, S., Sarma, Y. R. and Pant, R. P. (2003) Association of a badnavirus in black pepper (*Piper nigrum* L.) transmitted by mealybug (*Ferrisia virgata*) in India. *Current Science* **84**: 1547–1550.
- Bhat, A. I., Devasahayam, S., Venugopal, M. N. and Bhai, R. S. (2005) Distribution and incidence of viral diseases of black pepper in Karnataka and Kerala, India. *Journal of Plantation Crops* (in press)
- Dasgupta, I., Das, B. K., Nath, P. S., Mukhopadhyaya, S., Niazi, F. R. and Varma, A. (1996) Detection of rice tungro bacilliform virus in field and glasshouse samples from India using the polymerase chain reaction. *Journal of Virological Methods* **58**: 53–58.
- de Silva, D. P. P., Jones, P. and Shaw, W. W. (2002) Identification and transmission of *Piper yellow mottle virus* and *Cucumber mosaic virus* infecting black pepper (*Piper nigrum* L.) in Sri Lanka. *Plant Pathology* **51**: 537–545.
- Devasahayam, S. (2000) Insect pests. In: *Black Pepper Piper nigrum*, Ravindran, P. N. (Ed). Harwood Academic Publishers: Amsterdam, 309–334.

- Duarte, M. L. R., Albuquerque, F. C. and Chu, E. Y. (2001) New diseases affecting black pepper crop in Brazil. *International Pepper Bulletin* April–December: 51–57.
- Hull, R. (2002) *Matthews' Plant Virology*, 4th edn. Academic Press: New York, 1001.
- Lockhart, B. E. L., Kirtisak, K. A., Jones, P., Padmini, D. S., Olszewski, N. E., Lockhart, N., Nuarchan, D. and Sangalang, J. (1997) Identification of *Piper yellow mottle virus*, a mealybug-transmitted badnavirus infecting *Piper* spp in southeast Asia. *European Journal of Plant Pathology* **103**: 303–311.
- Roivainen, O. (1980) Mealybugs. In: *Vectors of Plant Pathogens*, Harris, K. F. and Maramorosch, K. (Eds). Academic Press: New York, 15–38.
- Sarma, Y. R., Kiranmai, G., Sreenivasulu, P., Anandaraj, M., Hema, M., Venkataramana, M., Murthy, A. K. and Reddy, D. V. R. (2001) Partial characterization and identification of a virus associated with stunt disease of black pepper (*Piper nigrum*) in South India. *Current Science* **80**: 459–462.
- Selvan, M. T. (2002) *Arecanut and Spices Database*, Directorate of Arecanut and Spices Development: Calicut, 117.
- Viswanathan, R., Alexander, K. C. and Garg, I. D. (1996) Detection of sugarcane bacilliform virus in sugarcane germplasm. *Acta Virologica* **40**: 5–8.

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## **Impact of coccinellid predator *Cheilomenes sexmaculata* (Fab.) on black citrus aphid *Toxoptera aurantii* (Boyer) infesting acid lime**

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**ABSTRACT:** Heavy population of black citrus aphid *Toxoptera aurantii* (Boyer) was observed along with the coccinellid predator *Cheilomenes sexmaculata* (Fab.) in January 2002 on acid lime at IIHR Farm, Bangalore. A mean number of 301.40 aphids per plant was noted. The number of *C. sexmaculata* ranged from 1.4 to 3.2 per plant. The aphids were completely cleared within two months. No other natural enemy was observed on the aphids. No significant changes in the abiotic factors were also noted. Hence the decline in the population of *T. aurantii* was attributed to the activity of *C. sexmaculata*. © 2005 Association for Advancement of Entomology

**KEYWORDS:** Acid lime, biological suppression, black citrus aphid, *Cheilomenes sexmaculata*, coccinellid, *Toxoptera aurantii*

The black citrus aphid, *Toxoptera aurantii* (Boyer) has been reported as a serious pest on several species of citrus including acid lime in India. Nymphs and adults infest tender shoots and leaves and suck the sap. They excrete large quantity of honeydew resulting in the development of sooty mould on leaves and shoots also. Repeated applications of chemical insecticides resulted in the outbreak of *T. aurantii* on citrus (Hussein and Kavar, 1985). Several natural enemies were reported on *T. aurantii* in India and elsewhere (Radhakrishnan and Muraleedharan, 1993; Dai and Dai, 1995). Natural enemies themselves if uninterrupted by insecticides help to check the aphid populations (Muraleedharan *et al.*, 1988). The present investigation was undertaken to determine the impact of *Cheilomenes sexmaculata* (Fab.) in the suppression of *T. aurantii* in the acid lime orchard.

The field study was conducted on five-year-old acid lime plants in Block No. 2 at IIHR Farm during January–March 2002. Fortnightly observations were initiated on 3rd January and continued up to 1st March 2002. The population of aphids and its predator, *C. sexmaculata* (both adults and grubs) were counted on 10 randomly selected aphid infested plants. In each plant, 4 shoots of 15 cm length were chosen for

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recording the observations. In each shoot, the number of aphids and *C. sexmaculata* were counted. A mean of 301.4 aphids per plant was observed on 3rd January 2002 and the aphid population declined to 134.6, 31.4 and 24.0 per plant on 17th January, 3rd and 28th February, respectively. Only *C. sexmaculata* was found feeding on *T. aurantii* throughout the study period. The population of *C. sexmaculata* ranged from 1.4 to 3.2 during January–February. The black citrus aphids were completely cleared in the first week of March 2002. According to Bhattacharyya and Dutta (1998), *T. aurantii* was regulated by coccinellids including *C. sexmaculata*. There were no significant changes in the abiotic factors like temperature, relative humidity and rainfall during the study period. Hence the decline in the population of *T. aurantii* was attributed to the activity of *C. sexmaculata*.

Mass production of *C. sexmaculata* has been standardized by Joshi *et al.* (2003). If *C. sexmaculata* does not appear in nature adequately, release of *C. sexmaculata* is suggested to suppress the black citrus aphid on citrus plants including acid lime.

#### REFERENCES

- Bhattacharyya, B. and Dutta, S. K. (1998) Black citrus aphid *Toxoptera aurantii* Boyer (Aphididae: Homoptera) in Assam. *Insect Environment* **3**(4): 109.
- Dai, Xuan and Dai, X (1995) Preliminary study on the ecological niches on the black citrus aphid and its natural enemies. *Journal of Tea Science* **15**(1): 79–80.
- Hussein, M. K. and Kwar, N. S. (1985) The role of aphid natural enemies in regulating population densities of *Toxoptera aurantii* (Homoptera: Aphididae) on citrus trees in Lebanon. *Arab Journal of Plant Protection* **3**(1): 11–17.
- Joshi, S., Prashanth Mohanraj, RabindraR. J. and RaoN. S. (2003) Production and use of coccinellids predators. *PDBC Technical Bulletin* No. 32 p 26.
- Muraleedharan, N., Selvasundaram, R. and Radhakrishnan, B. (1988) Natural enemies of certain tea pests occurring in Southern India. *Insect Science and its Applications* **9**(5): 647–654.
- Radhakrishnan, B. and Muraleedharan, N. (1993) Bio-ecology of six species of syrphid predators of the tea aphid. *Toxoptera aurantii* (Boyer de Fonscolombe) in Southern India. *Entomon* **18**: 175–180.

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## Field efficacy of commercial formulations of *Bacillus thuringiensis* var. *kurstaki* against *Papilio demoleus* L. on citrus

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**ABSTRACT:** Commercial formulations of *Bacillus thuringiensis* Berliner var. *kurstaki* (*Btk*), viz., Delfin, Halt, Dipel DF and Biobit were tested for their field efficacy against *Papilio demoleus* L. on citrus, at the Indian Institute of Horticultural Research farm, Bangalore. Results showed that five applications of *Btk* formulations @ 1 kg/ha effectively controlled the caterpillar population of *P. demoleus* L on citrus, when compared to untreated check. The mean larval population of 0.13–0.20 larva/plant recorded in *Btk* treated plants was on par with cypermethrin treated plants (0.20 larva/plant) and the unsprayed control plants recorded the maximum larval population of 3.03 larvae/plant. © 2005 Association for Advancement of Entomology

**KEYWORDS:** *Bacillus thuringiensis*, citrus, *Papilio demoleus*

The citrus caterpillar *Papilio demoleus* L. (Lepidoptera: Papilionidae) causes serious damage to citrus, especially in the nurseries and on newly flushed young plants in the field (Krishnamoorthy and Singh, 1986). We had observed an outbreak of this pest in 15-month-old citrus orchard at the Indian Institute of Horticultural Research (IIHR) farm, Bangalore, during September 2000. Earlier, Narayanan and Jayaraj (1974) studied the effectiveness of *Bacillus thuringiensis* Berliner var. *kurstaki* (*Btk*) on *P. demoleus* in the laboratory. Insecticides are used to control this pest, which often results in development of resistance and also killing of its natural enemies. Not much work has been done on the field efficacy of *Btk* against *P. demoleus* on citrus in India. Hence, the commercial formulations of *Btk*, viz. Dipel, Delfin, Biobit, Halt and Dipel DF were evaluated for their field efficacy against *P. demoleus* on citrus.

Field trial was laid out during September 2000 to assess the efficacy of the following commercial formulations of *Btk* compared to cypermethrin—Dipel DF, Biobit HPWP (Rallis India Ltd.), Halt WP (Wockhardt, Biotech Agri sciences), and Delfin WG (Sandoz India Ltd.) @ 1 kg/ha along with Triton x-100 (0.01%). Cypermethrin

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TABLE 1. Effect of *Btk* formulations against larval population of *Papilio demoleus* on citrus

| Treatment               | Larval count*       |                      |               |               |               | Mean           |
|-------------------------|---------------------|----------------------|---------------|---------------|---------------|----------------|
|                         | Before<br>the spray | Days after the spray |               |               |               |                |
|                         |                     | 7                    | 14            | 21            | 28            |                |
| Halt @ 1 g/lit.         | 2.8<br>(0.80)       | 0.0<br>(0.00)        | 0.2<br>(0.14) | 0.0<br>(0.00) | 0.3<br>(0.21) | 0.13<br>(0.09) |
| Delfin @ 1 g/lit.       | 2.3<br>(0.98)       | 0.0<br>(0.00)        | 0.5<br>(0.25) | 0.1<br>(0.07) | 0.2<br>(0.14) | 0.20<br>(0.14) |
| Biobit @ 1 g/lit.       | 2.6<br>(0.96)       | 0.1<br>(0.07)        | 0.0<br>(0.00) | 0.2<br>(0.14) | 0.3<br>(0.18) | 0.15<br>(0.13) |
| Dipel DF @ 1 g/lit.     | 2.9<br>(1.29)       | 0.0<br>(0.00)        | 0.2<br>(0.14) | 0.3<br>(0.21) | 0.3<br>(0.21) | 0.20<br>(0.14) |
| Cypermethrin @ 0.5 ml/l | 2.7<br>(1.17)       | 0.0<br>(0.00)        | 0.2<br>(0.14) | 0.3<br>(0.21) | 0.3<br>(0.21) | 0.20<br>(0.14) |
| Control                 | 2.9<br>(1.29)       | 3.3<br>(1.18)        | 3.6<br>(1.29) | 3.2<br>(1.29) | 2.0<br>(0.88) | 3.03<br>(1.16) |
| C.D. ( <i>P</i> = 0.05) | 0.60                | 0.30                 | 0.40          | 0.33          | 0.33          | 0.34           |

\*Mean of ten replications. (Figures in parenthesis are  $\sqrt{x} + 0.5$  transformed values.)

(Cymbush) was used @ 0.5 ml/l and an untreated check sprayed only with Triton x-100 (0.01%). There were ten replications per treatment (each plant constituted one replication) in a randomized design. *Btk* commercial formulations were applied at seven days intervals (total of 5 sprays) and cypermethrin at ten days interval (total of 3 sprays) from the time the caterpillars were noticed on the plant. Spraying was done with a knapsack sprayer using 700 l of spray fluid/ha. The spraying was carried out during evening hours to prevent the possible photo-inactivation of the pathogen.

Observations on the infestation were based on the number of caterpillars on ten whole plants for each treatment at weekly interval starting from first application. The larval counts were transferred into  $\sqrt{x} + 0.5$  as per Snedecor and Cochran (1957).

The initial mean larval count of *P. demoleus* before spray ranged from 2.3 to 2.9 larvae/plant (Table 1). The larval population was significantly brought down after the application of *Btk* formulations to 0.13–0.20 larvae/plant which was comparable to the insecticide. The control plants recorded significantly high larval population of 3.03 larvae/plant.

It is clear that any *Btk* formulation can be used for the control of *P. demoleus* on citrus. When the cost of the formulation is taken into consideration, Halt, which is indigenously produced, is cheaper than the other *Btk* formulations and can be recommended.

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#### REFERENCES

- Snedecor, and Cochran. (1957) *Statistical Methods*, Iowa State University Press: Ames, p 593.
- Narayanan, K. and Jayaraj, S. (1974) Mode of action of *Bacillus thuringiensis* Berliner in citrus leaf caterpillar, *Papilio demoleus* L.(Papilionidae: Lepidoptera). *Indian Journal of Experimental Biology* **12**: 89–91.
- Krishnamoorthy, A. and Singh, S. P. (1986) Record of the egg parasite, *Trichogramma chilonis* on *Papilio* spp. in citrus. *Current Science* **55**: 461.

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## Utilization of newer insecticides for management of spotted bollworm *Earias vittella* (Fabricius) (Lepidoptera: Noctuidae)

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**ABSTRACT:** Field studies were conducted to determine the efficacy of certain newer insecticides along with conventional insecticides against *Earias vittella* (Fab.) during *Kharif* 2002 and 2003 at the research farm of Indian Agricultural Research Institute, New Delhi, India. The newer insecticides, abamectin, emamectin benzoate, spinosad and betacyfluthrin properly protected the crop with minimum incidence of spotted bollworm in shed reproductives and green bolls during both the crop seasons, leading to increased seed cotton production of 164, 111.8, 110.4 and 125.7 per cent over control, respectively. The results suggest that the need-based application of these insecticides could be recommended as a component of sustainable management in cotton crop. © 2005 Association for Advancement of Entomology

**KEYWORDS:** cotton, *Earias vittella*, insecticides

*Earias vittella* (Fabricius), known as spotted bollworm of cotton as well as shoot and fruit borer of okra, is the most notorious pest of these two important Malvaceae crops, causing more than 40–50 per cent losses in different parts of India and hence its suppression is important (Mahapatro and Gupta, 1998). Though the distribution of this pest is restricted to cotton and okra growing areas, it causes heavy damage during vegetative stage of the crop onwards and is difficult to manage (Reed, 1994). Bollworms have defied conventional pest control methods and therefore are responsible for limiting the yield of seed cotton (Ulaganathan and Gupta, 2004). This study was carried out to determine the efficacy of new insecticides for control of spotted bollworm on cotton under field conditions and compare them with conventional insecticides.

Commercial formulations of the insecticides (Table 1) were obtained from the respective manufactures—emamectin benzoate (Proclaim, Syngenta, Crop Protection Limited, Mumbai), indoxacarb (Avaunt, EI Dupont India Pvt. Ltd., Gurgaon), cypermethrin (Cyperguard, DeNocil Crop Protection Limited, Mumbai), abamectin (Ver-

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timec, Syngenta Crop Protection Limited, Mumbai), spinosad (Tracer, DeNocil Crop Protection Limited, Mumbai), endosulfan (Thiodon, Aventis Crop Science, Mumbai), betacyfluthrin (Bulldock, Bayer (India) Life Science, Mumbai), chlorpyrifos (Nuchlor, EI DuPont India Ltd, Mumbai), lambda cyhalothrin (Karate, Zeneca Agrochemicals Limited, Chennai) and fipronil (Regent, Aventis Crop science, Mumbai).

Field trials were undertaken at the Indian Agricultural Research Institute, New Delhi, during cotton seasons of 2002 and 2003 (May–December) with American cotton (*Gossypium hirsutum* L. var. Pusa 8–6). The trials were laid in randomized block design with 11 treatments including control, replicated thrice. The crop was sown on second week of May in 2002 and last week of May during 2003 in 33.75 m<sup>2</sup> plots maintaining a spacing of 75 and 30 cm between rows and plants respectively. All the agronomic practices were followed for raising the crop under irrigated conditions. Since sucking pest population was below prescribed economic threshold level, no initial control measures were taken for these pests. However, to protect the crop from the attack of spotted bollworm, spraying was commenced with the setting of flowers in approximately 50% plants as suggested by Gupta and Agarwal (1985) and were repeated at regular intervals depending upon pest incidence. As such, a total of four rounds of spraying maximum at fortnightly intervals were given with high volume knapsack sprayer (spray fluid 400–750 litres/ha approx.) during both the years.

All the shed materials (squares, flowers, small bolls and big green bolls) from treated and untreated plots were collected and examined carefully for bollworm incidence. Fifteen green bolls were plucked from randomly selected plants in each plot preferably in the interior rows and dissected carefully for recording infestations on loculi basis and their larval populations, if any. Two pickings of cotton were done from each plot at the time of harvesting for calculating yield data. Seed cotton yield of both the pickings in each plot were added for computing total yield per hectare. The data thus were analysed using the statistical computer programme (Indostat Services<sup>®</sup>, Hyderabad).

All the insecticidal treatments were effective in suppressing the incidence of spotted bollworm and increasing the yield of seed cotton significantly (Table 1). Abamectin, emamectin benzoate, spinosad and betacyfluthrin were the most effective insecticides and reduced the infestation in shed reproductive and green bolls. In the field, emamectin benzoate is very effective in controlling lepidopterans like tobacco budworm and cotton bollworm at low concentrations (Dunbar *et al.*, 1998). Spinosad could also be used as an effective insecticide against target pest in appropriate resistance management programmes either alone or reinforced in mixture by other insecticides in order to avoid the selection of new cases of resistance (Ochou and Martin, 2003).

Maximum yield of seed cotton (2508.0, 2144.2, 2012.6 and 1999.3 kg/ha) was recorded from plots treated with abamectin @ 14.5 g ai/ha, beta cyfluthrin @ 18 g ai/ha, emamectin benzoate @ 9.8 g ai/ha and spinosad @ 75 g ai/ha respectively, followed by endosulfan @ 750 g ai/ha (1719.8 kg/ha) and cypermethrin @ 75 ai/ha (1656.6 kg/ha). Fipronil even @ 100g ai/ha was not effective and gave the least yield

TABLE 1. Efficacy of certain insecticides against *Earias vittella* in cotton (Pooled average data of 2002 and 2003)

| Insecticide              | dose<br>(g a.i./ha) | Per cent incidence of bollworms |                 |                 | Total yield<br>of seed<br>cotton<br>(kg/ha) | % increase<br>in yield<br>over<br>control |
|--------------------------|---------------------|---------------------------------|-----------------|-----------------|---|---|
|                          |                     | I                               | II              | III             |   |   |
| Abamectin 1.8 EC         | 14.5                | 49.4<br>(44.65)                 | 41.4<br>(40.04) | 27.3<br>(31.49) | 2508.0                                      | 164.0                                     |
| Beta-cyfluthrin 2.5 EC   | 18                  | 67.4<br>(55.18)                 | 55.6<br>(48.21) | 28.4<br>(32.20) | 2144.2                                      | 125.7                                     |
| Emamectin benzoate 5 WSG | 9.8                 | 80.1<br>(63.51)                 | 71.6<br>(57.79) | 30.1<br>(33.27) | 2012.6                                      | 111.8                                     |
| Spinosad 48 SC           | 75                  | 70.8<br>(57.29)                 | 77.8<br>(61.89) | 30.5<br>(33.52) | 1999.3                                      | 110.4                                     |
| Cypermethrin 10 EC       | 75                  | 88.5<br>(70.18)                 | 78.8<br>(62.36) | 25.8<br>(30.52) | 1656.6                                      | 74.4                                      |
| Endosulfan 35 EC         | 750                 | 69.2<br>(56.29)                 | 67.3<br>(55.12) | 33.9<br>(35.60) | 1719.8                                      | 81.0                                      |
| Lambda cyhalothrin 5 EC  | 25                  | 83.7<br>(66.19)                 | 82.2<br>(65.05) | 46.5<br>(42.99) | 1441.6                                      | 51.7                                      |
| Chlorpyrifos 20 EC       | 500                 | 78.7<br>(62.51)                 | 80.3<br>(63.65) | 32.3<br>(34.63) | 1314.8                                      | 38.4                                      |
| Indoxacarb 14.5 SC       | 75                  | 93.6<br>(75.37)                 | 89.6<br>(71.19) | 59.5<br>(50.47) | 1226.3                                      | 29.1                                      |
| Fipronil 5 SC            | 100                 | 95.5<br>(77.80)                 | 92.6<br>(74.23) | 49.5<br>(44.71) | 1190.8                                      | 25.3                                      |
| Untreated Control        |                     | 89.6<br>(85.20)                 | 88.0<br>(76.60) | 49.1<br>(53.85) | 950.1                                       | —   |
| SE <sub>m</sub> ±        |                     | 0.92                            | 0.60            | 1.06            | 49.57                                       | —   |
| CD ( $P \geq 0.05$ )     |                     | 2.74                            | 1.78            | 3.12            | 146.20                                      | —   |

Figures in parentheses are arc sine transformed values.

(1190.8 kg/ha). In control, the yield of seed cotton was 950.1 kg/ha. The percent increase in yield over control in the most effective treatments ranged from 74.4 to 164.0 (Table 1). Abamectin, emamectin benzoate, and spinosad are quickly absorbed into leaf tissue as demonstrated by Gary and Osborne (2001). Coupled with their efficacy against resistant pests, the newer insecticides can greatly reduce the number of sprays applied per season. Since growers have now a wide range of alternatives in the form of new and old chemicals, the best strategy would be to use effective compounds as one of the components of pest management tactics at need-based approach. It is possible to control *E. vittella* populations in cotton with lower rates of these novel insecticides. The outcome of these studies may also aid development of management programme for emerging pyrethroid-resistance in target cohort *E. vittella* populations.

#### REFERENCES

- Dunbar, D. M., Lawson, D. S., White, S. M., Ngo, N. and Dugger, P. (1998) Emamectin benzoate: control of the heliothine complex and impact on beneficial arthropods. In: *1998 Proceedings Beltwide Cotton Conferences*, San Diego: California, USA (January 5–9, 1998), vol. 2: 1116–1118.
- Gary, L. L. and Osborne, L. S. (2001) Chemical control of *Cactoblastis cactorum* (Lepidoptera: Pyralidae). *Florida Entomologists* **84**(4): 510–512.
- Gupta, G. P. and Agarwal, R. A. (1985) Effect of insecticides for the control of bollworms in relation to phenological characters in cotton. *Pesticides* **19**(10): 58–60.
- Mahapatro, G. K. and Gupta, G. P. (1998) Bio-potency test of some commercial formulations of *Bacillus thuringiensis* against spotted bollworm. *Earias vittella* Fab. *Pestology* **22**(8): 22–26.
- Ochou, O. G. and Martin, T. (2003) Activity spectrum of spinosad and indoxacarb: rationale for an innovative pyrethroid resistance management strategy in West Africa. *Resistant Pest Management Newsletter* **12**(2): 75–81.
- Reed, W. (1994) *Earias* spp. (Lepidoptera: Noctuidae). In: *Insects Pests of Cotton*, Mathews, G. A. and Tunstall, J. P. (Eds). Commonwealth Agricultural Bureau International: UK, 151–176.
- Ulaganathan, P. and Gupta, G. P. (2004) Effect of spray schedules on the control of bollworm complex of American cotton (*Gossypium hirsutum* L., var: Pusa 8–6). *Pesticide Research Journal* **16**(1): 23–27.

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## Allomonic effect of pigeon pea (*Cajanus cajan* (L.) Millsp.) plant extracts on egg parasitoid, *Trichogramma chilonis* Ishii parasitization

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**ABSTRACT:** The allomonic effect of pigeon pea plant extracts on the parasitization potential of *Trichogramma chilonis* Ishii was evaluated under both choice and nochoice conditions. The leaf and twig extracts exhibited strong antioviposition effects on *T. chilonis* and caused more than 50% reduction in parasitization over untreated check. This is one of the reasons why this egg parasitoid could not be exploited in pigeon pea. © 2005 Association for Advancement of Entomology

**KEYWORDS:** *Trichogramma chilonis*, allomonic effect, pigeon pea

*Trichogramma* is an important egg parasitoid used in many agricultural and horticultural crops to reduce pest populations. The parasitic activity of *Trichogramma* is mainly attributed to plant ecology through repellent/attractant properties. For example, the parasitization of *Helicoverpa armigera* (Hub) eggs by *T. pretiosum* Riley was relatively high on tomato but has only rarely been seen a chickpea (Romies and Shanower, 1996). Synomonal effect of pigeon pea plant extract on *T. brasiliensis* Ashmead and *T. japonicum* Ashmead has already been reported (Madhu *et al.*, 2000). Hence the present study was carried out to understand the sensitivity of the parasitoid *T. chilonis* to pigeon pea plant extracts.

Hundred g each of fresh leaves, flowers, apical twigs and pods of pigeon pea plants were collected from the field. They were chopped and stirred with 200 ml of hexane for 30 min and filtered through Whatman No. 1 filter paper. The extracts were distilled at 50 °C under reduced pressure for freeing of hexane. One g of extract was taken and mixed in 100 ml of hexane to have 1% concentration.

Approximately 400 *Corcyra cephalonica* eggs were pasted on an egg card (2 × 2 cm). Each card served as a replicate. There were four such replicates for each treatment. Egg cards were sprayed with one ml of plant extract using hand atomizer.

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TABLE 1. Effect of hexane extracts of pigeon pea plant parts on *T. chilonis* parasitization of *Corcyra* eggs

| Treatment*                  | Choice test                            |                               | % decrease over control | No-choice test                        |                         |
|-----------------------------|--|-------------------------------|-------------------------|---------------------------------------|-------------------------|
|                             | Percentage of eggs parasitized in 24 h |                               |                         | Percentage of eggs parasitized n 24 h | % decrease over control |
|                             | Treated %                              | Untreated %                   |                         | %                                     |                         |
| Leaf extract                | 22.19<br>(28.10) <sup>a</sup>          | 49.31<br>(44.61) <sup>b</sup> | −56.49                  | 24.19<br>(29.46) <sup>d</sup>         | −65.66                  |
| Twig extract                | 21.63<br>(27.71) <sup>d</sup>          | 49.81<br>(44.89) <sup>b</sup> | −57.59                  | 24.56<br>(29.71) <sup>d</sup>         | −66.91                  |
| Flower extract              | 36.38<br>(37.10) <sup>b</sup>          | 51.19<br>(45.68) <sup>a</sup> | −28.67                  | 44.75<br>(41.99) <sup>b</sup>         | −36.47                  |
| Pod extract                 | 33.81<br>(35.56) <sup>c</sup>          | 50.00<br>(45.00) <sup>b</sup> | −33.71                  | 39.31<br>(38.33) <sup>c</sup>         | −44.36                  |
| Hexane<br>(untreated check) | 51.00<br>(45.43) <sup>a</sup>          | 51.94<br>(46.11) <sup>a</sup> | —                       | 70.44<br>(57.07) <sup>a</sup>         | —                       |

\*The extracts were sprayed at 1% concentration over 400 eggs on an egg-card. There were 4 replications for each treatment.

Figures in parentheses are arc sine transformed values.

Means followed by the same alphabet are not significantly different ( $P = 0.05$ ) by DMRT.

Hexane treated egg card served as untreated check. Five pairs of freshly emerged *T. chilonis* adults were introduced into each test tube (15 cm long and 2 cm dia) with the help of a fine camel hairbrush. Streaks of diluted honey (50%) were provided on the inner walls of the specimen tube as food for adults. In the nochoice test, five pairs of females were provided with cards bearing only treated eggs whereas in the choice test, cards bearing 400 treated and 400 untreated eggs were given. Tubes were plugged with cotton wads. Adults were removed after 24 h of exposure to eggs. Data on the level of parasitism both in the choice and nochoice tests were recorded five days after parasitisation. The parasitized eggs were distinguished from unparasitized eggs by blackening of the former on the fifth day (Raguraman and Singh, 1999).

The host eggs treated with one per cent hexane extracts of pigeon pea leaves, twigs, flowers and pods significantly hindered the parasitization by *T. chilonis*. The decrease in parasitization by *T. chilonis* from untreated check ranged from 28.67 to 57.59 per cent in choice condition and 36.47 to 66.91 per cent in no-choice condition. The result clearly shows that pigeon pea plant extracts caused upto 50 per cent reduction in parasitization by *T. chilonis* (Table 1)

Of the four plant parts tested, the leaf and twig extracts showed more negative influence than flower and pod extracts on parasitization by *T. chilonis*. The differences in the sensitivity of the parasitoid to these extracts may possibly be due to the presence of secondary metabolites in the plant. Romies and Shanower (1996) showed that on pigeon pea, the parasitoids are repelled on or near the plant surface and walking behaviour was found to be significantly hindered by trichomes and trichomal

exudates on pigeon pea buds and pods. Bhatnagar and Davies (1981) found that in traditional pigeon pea-sorghum intercropping systems in India, where pigeon pea produces flowers at least one month after sorghum anthesis, *Trichogramma* spp. were found to parasitize only at low level on *H. armigera* eggs on the pigeon pea. However, Madhu *et al.* (2000) reported synomonal effects of pigeon pea plant extracts to both *T. brasiliensis* (Ashmead) and *T. japonicum* (Ashmead) due to the presence of tricosane in these extracts. Hence, further investigations should be focussed on the secretions and the chemical ecology in pigeon pea responsible for the negative effect on this potential parasitoid.

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#### REFERENCES

- Bhatnagar, V. S. and Davies, J. C. (1981) Pest management in intercrop subsistence farming. In: *International workshop on Intercropping*, ICRISAT: Patancheru, Andhra Pradesh, 249–257.
- Madhu, S., Paul, A. V. N. and Singh, D. B. (2000) Synomonal effect of different plant extracts on parasitism by *Trichogramma brasiliensis* (Ashmead). *Shashpa* 7: 35–40.
- Raguraman, S. and Singh, R. P. (1999) Biological effect of neem (*Azadirachta indica* A. Juss) seed oil on egg parasitoid, *Trichogramma chilonis*. *J. Econ. Entomol.* 2: 1274–1280.
- Romies, J. and Shanower, T.G. (1996) Arthropod natural enemies of *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae) in India. *Biocontrol Science and Technology*. 6: 481–508.

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## Occurance of *Ochlerotatus (Mucidus) Laniger* (Wiedemann) (Diptera: Culicidae) in Assam, India

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**ABSTRACT:** The communication reports the first confirmed occurrence of *Ochlerotatus (Mucidus) laniger* (Wiedemann) in forest areas of Assam, north-east India.

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**KEYWORDS:** *Ochlerotatus laniger*, new distribution record, Assam, India

*Ochlerotatus* Lynch Arribalzaga was earlier a subgenus of genus *Aedes* Meigen that was later elevated to the generic rank and the subgenus *Mucidus* Theobald was shifted from genus *Aedes* to *Ochlerotatus* (Reinert, 2000). Tyson (1970) mentioned 15 recognised species belonging to *Ochlerotatus (Mucidus)*— three from Australasian region, six from Oriental region and six from Ethiopian region. *Ochlerotatus (Mucidus) laniger* (Wiedemann), the Oriental species, was recorded in Thailand, Philippines, West Malaysia, Singapore, Indonesia and South Vietnam (Tyson, 1970). Barraud (1934) examined two unlabelled male specimens of *Oc. (Muc.) laniger* in Malaria Survey of India collections and speculated them to be probably from Assam, India. Further, the confirmed identification of *Oc. laniger* had some problems (Knight, 1947; Knight and Hull, 1951) which was later solved by Mattingly (1961). Assam (eastern India) was not included as a distribution locality for this species in the world mosquito catalogue of Knight and Stone (1977).

Recently (May–June 2003), during collection of *Anopheles (Cellia) dirus* Peyton and Harrison immatures, as a part of our ongoing study on its ecology, three larvae were collected from shaded ground pools, one from Nambor forest range in Golaghat district and two from Soraipung forest range in Dibrugarh district, Assam, India. Both these localities are part of evergreen rain forests. These larvae were link reared individually in plastic photo vials in the laboratory from which one male and two females emerged. The adult, larval and pupal characters of the emerged specimens, on examination, were found matching with the description of *Oc. laniger* provided

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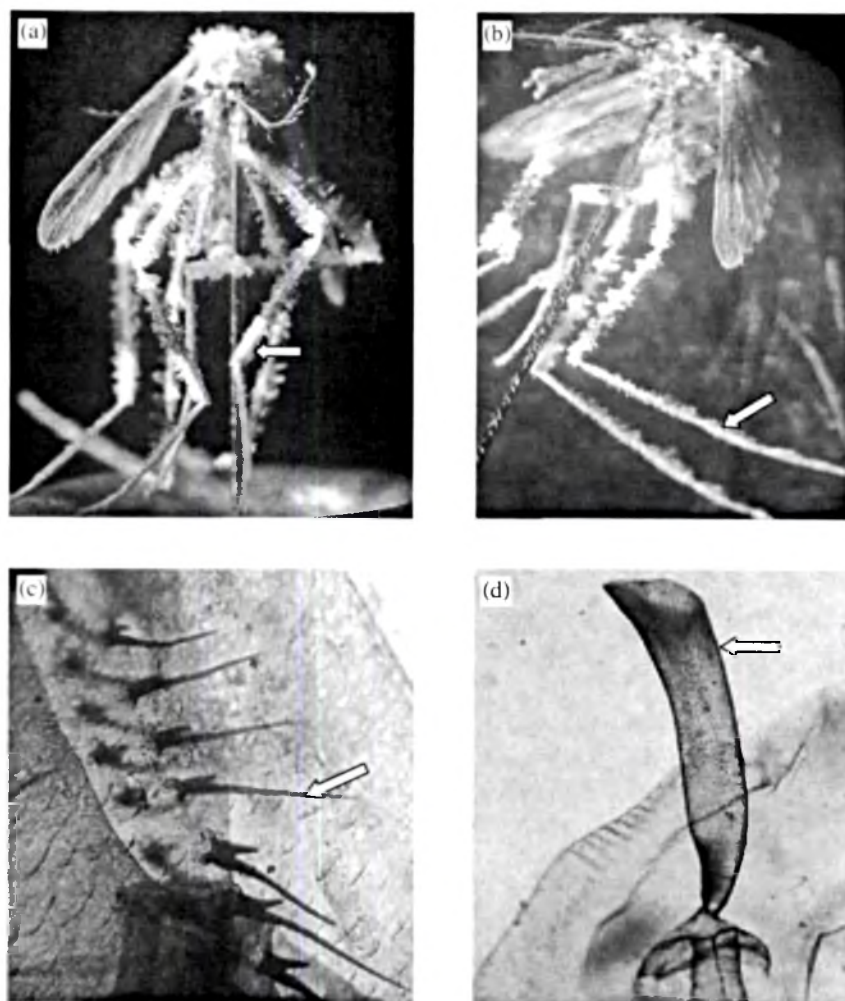


FIGURE 1. The adult, larval siphon and pupal trumpet of *Ochlerotatus (Mucidus) laniger*.

by Tyson (1970). The adults possessed the diagnostic characters of apical white band on fore tibia longer than 0.25 of total tibial length (Fig. 1a) along with brown hind tarsomere II (Fig. 1b). The larvae closely resembled that of *Ochlerotatus (Mucidus) scatophagoides* (Theobald) in having elongated apices of the pecten scales (Fig. 1c), a feature that differentiates these two from other Oriental *Mucidus* species. However, the trumpet of pupa was moderately long and slender (Fig. 1d) that easily distinguishes it from the short and thick trumpet of *Oc. scatophagoides*. The adult specimens along with their associated larval and pupal exuviae are kept in the museum (specimen no

A-1864, A-1865; I-921, I-922; p-906, p-907) of Regional Medical Research Centre, NE Region (Indian Council of Medical Research), Dibrugarh, Assam, India.

Tyson (1970) remarked that distribution record of mosquitoes belonging to genus *Ochlerotatus* subgenus *Mucidus* presents interesting features like presence of African species *Ochlerotatus (Mucidus) tonkingi* Gebert on Mauritius, absence of *Mucidus* mosquitoes on Madagascar and the islands of the Seychelles-Mauritius Ridge and close relationship of the African species *Ochlerotatus (Mucidus) sudanensis* (Theobald), the Oriental species *Oc. scatophagoides* and the Australasian species *Ochlerotatus (Mucidus) alternans* (Westwood).

Present report is the first confirmed report of the occurrence of *Oc. laniger* in India and supports the Barraud's (1934) speculation of its presence in Assam, India.

The larvae were collected from fresh rain fed ground pools that were completely shaded, having rotten leaves at the bottom, breeding in association with *Aedes (Paraedes) ostentatio* (Leicester), *An. (Cel.) dirus* Peyton and Harrison, *Culex (Lophoceraomyia) mammalifer* (Leicester) and *Verrallina (Neomacleaya) rami* (Barraud). The *Oc. (Mucidus)* mosquito species are not yet recognized as vector of any human pathogen but many species are known to feed on humans (Tyson, 1970).

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#### REFERENCES

- Barraud, P. J. (1934) The fauna of British India including Ceylon and Burma (Diptera Vol. V) Family Culicidae. In: *Tribes Megarhinini and Culicini*, Taylor and Francis: London, 1-463.
- Knight, K. L. (1947) The *Aedes (Mucidus)* mosquitoes of the pacific (Diptera: Culicidae). *Journal of the Washington Academy of Sciences*. **37**: 315-325.
- Knight, K. L. and Hull, W. B. (1951) The *Aedes* mosquitoes of the Philippine islands. I Keys to the species. Subgenera *Mucidus*, *Ochlerotatus*, and *Finlaya* (Diptera: Culicidae). *Pacific Sciences*. **5**: 211-251.
- Knight, K. L. and Stone, A. (1977) *Catalog of the Mosquitoes of the World (Diptera: Culicidae)*, The Geo. W. King Company Baltimore: Maryland, 1-611.
- Mattingly, P. F. (1961) In: *The Culicine Mosquitoes of the Indomalayan Area. Part V*, Genus *Aedes* Meigen, subgenus *Mucidus* Theobald, *Ochlerotatus* Lynch Arribalzaga and *Neomelaniconeion* Newstead. British Museum of Natural History: London. 1-62.
- Reinert, J. F. (2000) New classification for the composite genus *Aedes* (Diptera: Culicidae: Aedini), elevation of subgenus *Ochlerotatus* to generic rank, reclassification of the other subgenera, and notes on certain subgenera and species. *Journal of the American Mosquito Control Association*. **16**: 175-188.
- Tyson, W. H. (1970) Contributions to the Mosquito Fauna of Southeast Asia. VII Genus *Aedeomyia* Theobald in Southeast Asia. VIII. Genus *Aedes*, subgenus *Mucidus* Theobald in Southeast Asia. *Contributions of the American Entomological Institute*. **6(2)**: 1-80.

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## **Insects: Structure, function and Biodiversity**

**By Dunstan P. Ambrose, Kalyani Publishers, Ludhiana  
821 pp Rs. 550/- (2004)**

In recent years Entomology has received greater attention and several books are being published, with emphasis on diverse aspects, providing overall information on the structure and function of insects. The book under review is unique in several respects, typically suited as a text book at different levels, providing adequate information on the structure and function as well as the diversity of insects, in many instances introducing reduviids as example, in tune with the author's specialization. Essentially the volume is divided into five main sections: Structure and function, Behaviour, Biosystematics, Ecology and Experimental Entomology and comprising 22 chapters. Quite some emphasis has been laid on structure and function supported by excellent illustrations. Biosystematics has been dealt with in a lucid way providing the basic characters of diverse taxa, facilitating easy understanding. What is striking is the quality of the illustrations, which well support the descriptions provided. Compared to the first three sections, that on Ecology has restricted exposition presumably wanting to reduce the bulkiness of the volume. At the same time the essential aspects of this field have not been lost sight of and perhaps greater emphasis is one to be laid in the succeeding editions. What is notable is the inclusion of the section on Experimental Entomology, which is overlooked in most textbooks. This is a very useful chapter to students of all categories. The long list of references under each chapter and the meaningful glossary add to the quality of the book.

Dr. Ambrose has toiled hard on the production of this excellent book for some years to be able to come out with a good textbook on Entomology. Needless to say that this excellent volume has to find a place in the shelves of every reader, both student and teacher.

Chennai  
1-2-2005

Prof. T. N. Ananthakrishnan  
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